# Synthesis of *Carbon*-Analogues of 2,4-Diaminopyrimidine and 2-Oxo-4-Aminopyrimidine Homo-DNA Nucleosides

# Study of Homo-DNA Oligonucleotides Containing *Carbon*-Analogues of the 2,4-Diaminopyrimidine Nucleotide

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**zur**

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## PUBLIKATIONEN UND PRÄSENTATIONEN

M. Löpfe, B. Bischof, J. S. Siegel, **Synthesis and structure of homo-DNA-*C*-nucleosides**, Manuskript in Bearbeitung.

M. Löpfe, J. S. Siegel, **Diastereoselective Synthesis of 2',3'-Dideoxy-$\beta$-*C*-Glucopyranosides as Intermediates for the Synthesis of 2',3'-Dideoxy-$\beta$-D-Glucopyranosyl-*C*-Nucleosides**, *Nucleosides, Nucleotides and Nucleic Acids* **2007**, *26*(8/9), 1029-1035.

M. Löpfe, J. S. Siegel, **Homo-DNA-*C*-Nucleosides,** Poster präsentiert am *Nucleic Acid Chemical Biology (NACB) Symposium*, University of Southern Denmark, Odense, Dänemark, 06/2007.

M. Löpfe, J. S. Siegel, **Einfluss von Veränderungen der Nucleobasen und des Zuckerrückgrates auf die Duplexstabilität in DNA-analogen Systemen,** Poster präsentiert am *Symposium zum Forschungskredit der Universität Zürich*, Universität Zürich, Schweiz, 06/2007.

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M. Löpfe, J. S. Siegel, **Synthesis of Homo-DNA-*C*-nucleosides**, Poster präsentiert am *Swiss Chemical Society – Fall Meeting*, Universität Zürich, Schweiz, 10/2006.

Weitere Beiträge wurden an folgenden Symposien geleistet:

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ABSTRACT OF THE DISSERTATION

Synthesis of *Carbon*-Analogues of 2,4-Diaminopyrimidine and 2-Oxo-4-Aminopyrimidine Homo-DNA Nucleosides

Study of Homo-DNA Oligonucleotides Containing *Carbon*-Analogues of the 2,4-Diaminopyrimidine Nucleotide

by

Michael Löpfe

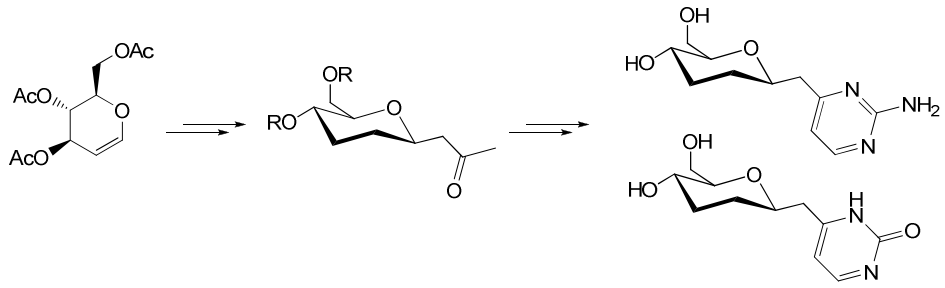
University of Zurich, 2008

Professor Jay S. Siegel, Chair

This doctoral work is divided into two sections: the first part deals with the synthesis of carbon-analogues of 2,4-diamonpyrimidin homo-DNA nucleosides and 2-oxo-4-aminopyrimidin homo-DNA nucleosides. In the second part homo-DNA-oligonucleotides containing carbon-analogues of the 2,4-diaminopyrimidine homo-DNA nucleoside were investigated.

Starting from known stabilities of exocyclic amino nucleosides (EAN's) a model compound was designed to increase the stability of these compounds. By replacing the exocyclic nitrogen by a carbon the unstable aminohemiacetal was replaced by a stable cyclic ether. Due to the fact that EAN's undergo a furanose-pyranose isomerization and the pyranose is thermodynamically favored species, a pyranoside analogue of DNA, the homo-DNA was evaluated. In the first part of the thesis a series of homo-DNA-carbon-nucleosides were prepared from tris-*O*-acetyl-D-glucal. The key step of the synthesis was the diastereoselective

preparation of the $\beta$-*C*-glycoside, as shown below. The $\beta$-*C*-glycoside was accessible by a rearrangement from the $\alpha$-*C*-glycoside under *Lewis* acidic conditions.




In the second part of the thesis the carbon-analogue of the 2,4-diaminopyrimidine-homo-DNA-nucleoside was incorporated by solid phase synthesis in homo-DNA-oligonucleotides. It was shown that an adenine(A)-2,4-diaminopyrimidine(D) exchange near the 4' end in the self complementary octamer ddGlc[UX$(UA)_3$] did not result in a enthalpic destabilization of the *Watson-Crick* A-U base pairing ($\Delta$H -165 kJ/mol (D); -163 kJ/mol (A)). The entropic destabilization was explained by the increase of rotational degrees of freedom of the D-nucleoside compared to the A-nucleoside. An exchange of an A nucleoside in a central position of a selfcomplementary homo-DNA oligonucleotide with the general formula ddGlc[$(UA)_n$] resulted in a much stronger influence on the homo-DNA duplex stability compared to the A-D exchange near the 4' end: For the 14-mer ddGlc[$(UA)_3$UD$(UA)_3$] an inter- or intramolecular duplex formation was found dependant on the oligonucleotide concentration.

An investigation of an A-D exchange on the stability of the reverse *Hoogsteen* A-A pairing resulted in no enthalpic destabilization by an A-D exchange: Compared to the octamer ddGlc[$A_8$] even an enthalpic stabilization was found if two reverse *Hoogsteen* A-A base pairings were replaced by two A-D base pairings ($\Delta$H -208 kJ/mol (ddGlc[$A_8$]); $\Delta$H -322 kJ/mol ddGlc[$A_2DA_5$]), although the enthalpic stabilization was overcompensated by an

entropic destabilization (ΔS -520 J/(mol*K) (ddGlc[$A_8$]); ΔS -917 J/(mol*K) ddGlc[$A_2DA_5$]). Also in the nonamer ddGlc[$UD_8$], in which all A nucleosides were replaced against D nucleosides, an interaction was still detectable.

Further investigations were performed with non selfcomplementary oligonucleotides of the general formula ddGlc[CAUA-$X^1$-GUGA] und ddGlc[UCAC-$X^2$-UAUG]. Comparisons of the *Watson-Crick* A-U ($X^1$ = A; $X^2$ = U) base pairing with the corresponding D-U base pairing ($X^1$ = D, $X^2$ = U) were performed. Further studies were performed to study the influence of a A-D exchange in the following base pairings A-A ($X^1$ = A; $X^2$ = A to $X^1$ = D; $X^2$ = A); A-C ($X^1$ = A; $X^2$ = C) and A-G ($X^1$ = A; $X^2$ = G). All these investigations resulted in a enthalpic destabilization and entropic stabilization of the duplex formation.

# ZUSAMMENFASSUNG DER DISSERTATION

Synthese von Kohlenstoff-Analogen des 2,4-Diaminopyrimidin und 2-Oxo-4-Aminopyrimidin Homo-DNA Nukleosides.

Untersuchung von Homo-DNA Oligonukleotiden welche ein Kohlenstoff-Analogon des 2,4-Diaminopyrimidin beinhalten

von

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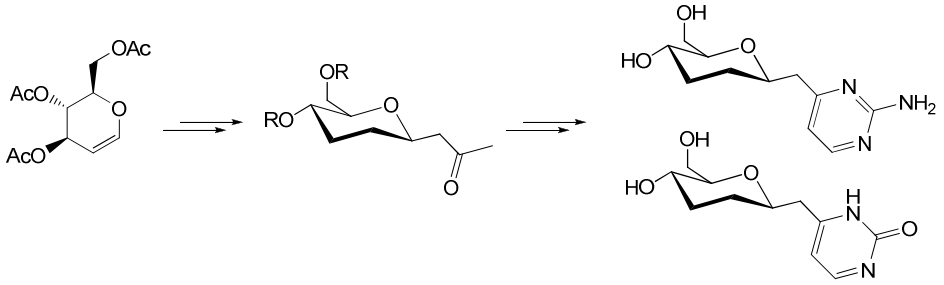
Universität Zürich, 2008

Prof. Dr. Jay S. Siegel, Vorsitz

Die vorliegende Doktorabeit ist in zwei Abschnitte gegliedert: Die Synthese von Kohlenstoff-Analogen von 2,4-Diaminopyrimidin homo-DNA Nukleosiden und 2-Oxo-4-aminopyrimidin homo-DNA Nukleosiden einerseits sowie die Untersuchung von Homo-DNA-Oligonukleotiden, die das Kohlenstoff-Analogon des 2,4-Diaminopyrimidin homo-DNA Nukleosids beinhalten.

Ausgehend von bekannten Untersuchungen zu Stabilitäten von exozyklischen Aminonukleosiden (EAN's) wurde eine Modellverbindung entworfen, in welcher ein für die Instabilität der Verbindungen verantwortlicher Stickstoff durch ein Kohlenstoff ersetzt wird und somit die Aminoacetalgruppe durch einen stabilen cyclischen Ether ersetzt wurde. Aufgrund der Tatsache dass EAN's zu einer Furanose-Pyranose Isomerisierung tendieren und im thermodynamischen Gleichgewicht bevorzug als Pyranose vorliegen, wurde ein pyranosides Analogon der DNA, die sogenannte homo-DNA untersucht. Im ersten Teil der Arbeit wurde ausgehend von Tris-*O*-acetyl-D-glucal eine Serie von homo-DNA-*C*-

Nukleosiden hergestellt. Der zentrale Punkt der Synthese war die diastereoselektive Herstellung des $\beta$-*C*-Glycosids, das durch eine *in situ* Umlagerung vom $\alpha$-C-Glycosid unter *Lewis*-Säurenkatalyse erhalten wurde.




Im zweiten Teil der Arbeit wurde das gemäss obiger Synthese erhaltene Kohlenstoff Analogon des 2,4-Diaminopyrimidin-homo-DNA Nukleosids (D) mittels Festphasensynthese in homo-DNA Oligonukleotide eingebaut. Es wurde gezeigt, dass im selbstkomplementären Oktamer ddGlc[UX$(UA)_3$] der Einfluss eines Adenin(A)-D Austausches nahe des 4‘-Endes des Oligos zu keiner enthalpischen Destabilisierung der *Watson-Crick* Basenpaarung führt ($\Delta$H -165 kJ/mol (D); -163 kJ/mol (A)). Eine gefundene entropische Destabilisierung erklärt sich durch erhöhte Rotationsfreiheitsgrade des D-Nukleosids gegenüber dem A-Nukleosid. Durch eine Integration des D-Nukleosids in die Mitte eines selbstkomplementären Oligonucleotides und somit das Aufeinandertreffen zweier U-D Basenpaarungen erfolgt eine drastische Beeinflussung der Duplexstabilität: Für das selbstkomplementäre 14-mer ddGlc[$(UA)_3$UD$(UA)_3$] wurde eine von der Oligonucleotidkonzentration abhängige inter- bzw. intramolekulare Duplexbildung gefunden.

Eine Untersuchung eines A-D Austausches auf die Stabilität der reverse *Hoogsteen* A-A Paarung zeigte, dass auch diese Baasenpaarung keine enthalpische Destabilisierung durch einen A-D Austausch erfährt: Gegenüber dem Oktamer ddGlc[$A_8$] trat beim Austausch zweier A-A gegen A-D Paarung eine enthalpische Stabilisierung auf ($\Delta$H -208 kJ/mol (ddGlc[$A_8$]);

ΔH -322 kJ/mol ddGlc[$A_2DA_5$]), welche allerdings durch eine entropische Destabilisierung überkompensiert wurde (ΔS -520 J/(mol*K) (ddGlc[$A_8$]); ΔS -917 J/(mol*K) ddGlc[$A_2DA_5$]). Zusätzlich wurde gezeigt, dass selbst im extremen Fall des ddGlc[$UD_8$], in welchem alle A gegen D ausgetauscht wurden, noch eine Interaktion auftritt.

Weitere Untersuchungen nicht selbstkomplementärer Oligonucleotide der allgemeinen Form ddGlc[CAUA-$X^1$-GUGA] und ddGlc[UCAC-$X^2$-UAUG] wurden durchgeführt. Dabei wurde sowohl ein Vergleich eines *Watson-Crick* A-U ($X^1$ = A; $X^2$ = U) Basenpaares mit dem ensprechenden D-U ($X^1$ = D, $X^2$ = U) wie auch ein A-D Austausch in den folgenden Basenpaarungen A-A ($X^1$ = A; $X^2$ = A zu $X^1$ = D; $X^2$ = A); A-C ($X^1$ = A; $X^2$ = C) sowie A-G ($X^1$ = A; $X^2$ = G) untersucht. Diese Untersuchungen resultierten alle in einer enthalpischen Destabilisierung und einer entropischen Stabilisierung der Duplexbildung bei einem A-D Austausch.

# 1. Introduction

„Wir wollen nicht nur wissen *wie* die Natur ist (und *wie* ihre Vorgänge ablaufen), sondern wir wollen auch nach Möglichkeit das vielleicht utopisch und anmassend erscheinende Ziel erreichen, zu wissen, warum die Natur *so und nicht anders ist*" [Albert Einstein].[1] [We not only want to know how nature is (and *how* her processes are carried through), but also want to reach, if possible, a goal which may seem utopian and presumptuous, namely, to know why nature *is such and not otherwise*].

## *1.1 Biological functions of nucleosides, nucleotides and nucleic acids*[2]

Nucleic acids were first isolated in 1869 by *Miescher* in *Hoppe-Seyler*'s laboratory at the University of Tubingen, Germany, from the nuclei of leukocytes found on discarded surgical bandages,[3] and the presence of nucleic acids in other cells was demonstrated within a few years. The tetranucleotide hypothesis[4] by *Levene* was only widely accepted in the 1930s and 1940s. This hypothesis predicted that nucleic acids have a monotonously repeating sequence of all four bases and were, therefore, not suspected of having a genetic function.

In 1928, *Griffith*, a British medical officer made a startling discovery: injecting a mixture of heat-killed virulent S pneumococci (*Diplococcus pneumoniae*) and non-pathogenic R pneumococci in mice resulted in death of most of the mice.[5] S pneumococci were found in the blood of the dead mice, indicating a transformation from the otherwise innocuous R pneumococci to the S form, induced by the coinjected heat-killed S pneumococci. However, the nature of the transforming principle remained unclear.

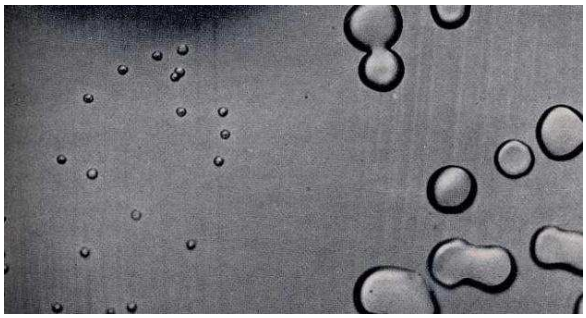


**Fig. 1** *Avery*'s pneumococci. Large glistening colonies are virulent S-type pneumococci resulting from transformation of non-pathogenic R-type pneumococci (smaller colonies) by DNA from heat-killed S pneumococci.[6]

In 1944, *Avery*, *MacLeod*, and *McCarty* reported that the transforming principle is DNA.[6] The conclusion was based on the observation that the transforming principle had all the physical and chemical properties of DNA, contained no detectable protein, was unaffected by enzymes that catalyze the hydrolysis of proteins and RNA, but was totally inactivated by treatment with an enzyme that catalyzes the hydrolysis of DNA.

*Chargaff*, at the end of the 1940s, refuted the tetranucleotide hypothesis by accurate determination of DNA base ratios and thereby indicated that DNA could be a complex molecule that is a requirement for genetic functions. [7]

*Brinster* demonstrated in 1982 that eukaryotes are also subject to transformation by DNA.[8] By microinjection of DNA bearing the gene for rat growth hormone into the nuclei of fertilized mouse eggs and implantation of these eggs into the uteri of foster mothers. The resulting transgenic mice had high levels of rat growth hormone in their serum and grew to nearly twice the weight of their normal littermates.

Today it is known that nucleotides are biologically ubiquitous substances. Besides their central role in both the storage and expression of genetic information as monomeric units of nucleic acids, they participate in many biochemical processes:[2]

1.) Nucleoside triphosphates (especially ATP) are the end products of the majority of energy-releasing pathways and the substances whose utilization drives most energy-requiring processes. [9]

2.) Many pathways are partially regulated by the levels of nucleotides (ATP, ADP) and certain nucleotides function as intracellular signals that regulate the activities of numerous metabolic processes.[10]

3.) In many enzymatic reactions nucleotide derivatives, such as nicotinamide adenine dinucleotide, [11] flavin adenine dinucleotide, or coenzyme A, are required participants.

4.) As components of the ribozymes, nucleotides are catalytically active.[12]

## *1.2 DNA structure*

### 1.2.1 Nucleosides and nucleotides[2,13]

Nucleic acids consist of a linear array of monomers, called nucleotides. Nucleotides are the phosphate esters of nucleosides. The phosphate group may be bonded to the C5'- (5'-nucleotide) or to the C3'-hydroxygroup (3'-nucleotide). Nucleotides are constructed of three components: a nitrogen-containing heterocyclic base (major purines: adenine (A) and guanine (G), major pyrimidines: cytosine (C), uracil (U) (U occurs mainly in RNA) and thymine (T) (5-methyluracil, occurs mainly in DNA), a pentose sugar (D-ribose in RNA, 2-deoxy-D-ribose in DNA), and a phosphate residue. The pentose is locked into a five-membered furanose ring by the bond from the C1' of the sugar to N1 of C, U, T or to N9 of A or G. This bond is on the same side of the sugar ring as the C4'-C5' bond and is defined as a $\beta$-glycosylic linkage (vs. $\alpha$-glycosylic linkage with the C4'-C5' bond and the nucleobase on different sides of the sugar ring) (**Fig. 2**).

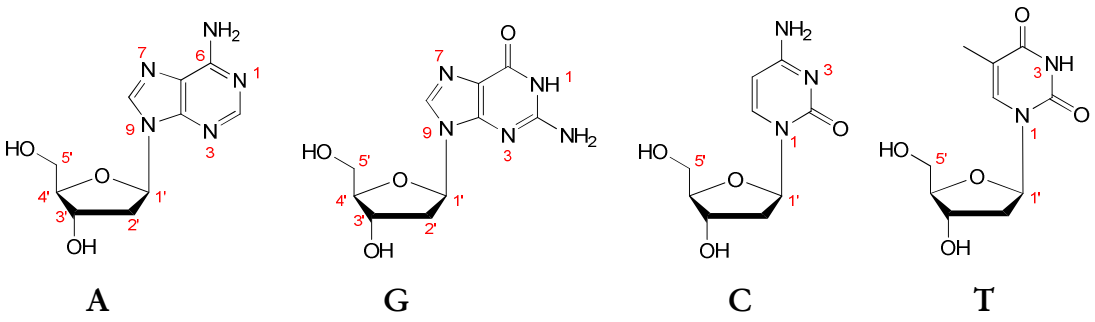



**Fig. 2** Structures of the four major deoxynucleosides found in DNA: deoxyadenosine (A), deoxyguanosine (G), deoxycytidine (C) and deoxythymidine (T) in their dominant tautomeric forms with the IUPAC numbering systems for purines, pyrimidines and the 2-deoxy-D-ribose.

## 1.2.2 Physical properties of nucleosides and nucleotides

### 1.2.2.1 Ionization[13]

The acid-base behavior of nucleotide is an important physical characteristic, because the acid-base behavior determines the charge and also the tautomeric structure which is responsible for the donation and acceptance of hydrogen bonds (**Table 1**).

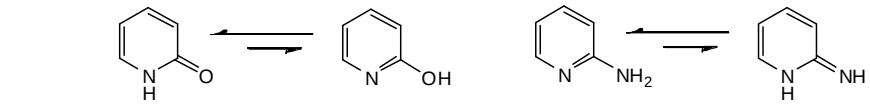
| Base (site of protonation) | Nucleoside | 3'-Nucleotide | 5'-Nucleotide |
|---|---|---|---|
| Adenine (N1) | 3.5 | 3.7 | 3.9 |
| Cytosine (N3) | 4.2 | 4.4 | 4.6 |
| Guanine (N7) | 3.3 | - | - |
| Guanine (N1) | 9.4 | 9.8 | 10.0 |
| Thymine (N3) | 9.9 | - | 10.5 |
| Uracil (N3) | 9.4 | 10.0 | 10.1 |

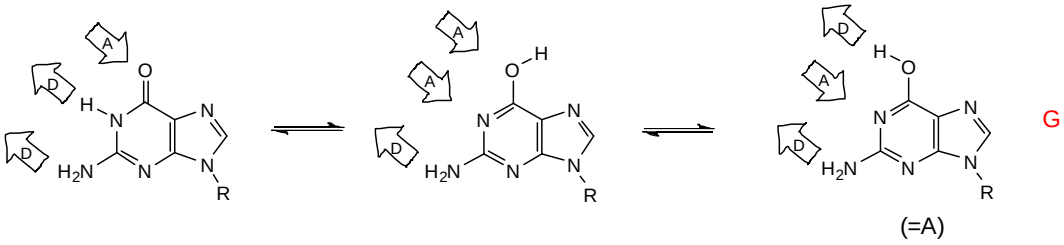
**Table 1** *pKa* values for bases in nucleosides and nucleotides (concentration $5^{-5}$ M to $10^{-5}$ M, data relate to 20° C and zero salt concentration)[14]

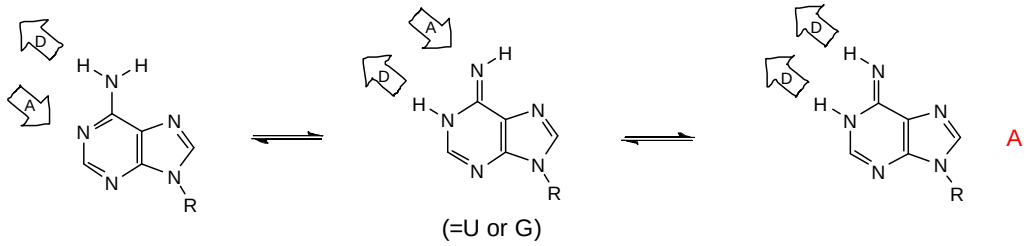
All of the bases are uncharged in a pH range of 5-9. The nucleotide phosphates possess pKa values of around 1 and 7 (in the case of the monoester) for the first and second deprotonation, respectively. The three amino bases, A, C, and G, become protonated on one of the ring nitrogens rather than the exocyclic amino group.[15]

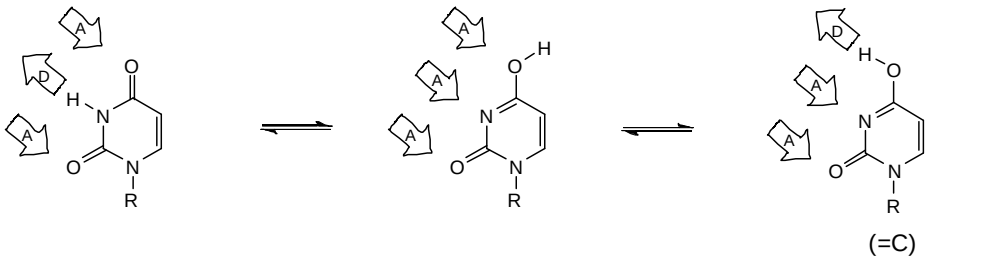
### 1.2.2.2 Tautomerism of bases

Heterocyclic molecules in solution frequently yield a mixed population of species in rapid equilibrium when hydrogen atoms attached to nitrogens are able to migrate to other, free nitrogens or to keto oxygens within the same molecule.[16] This kind of tautomerism depends mostly on the dielectric constant of the solvent and the *pKa* of the respective heteroatoms.[17] By tautomeric changes, uracil and guanine, in the enol form, simulate cytosine and adenine, and cytosine and adenine in the imino form may substitute for uracil and guanine (**Fig. 3**).


keto
enol
amine
imine


G
(=A)


A
(=U or G)


(=C)
U

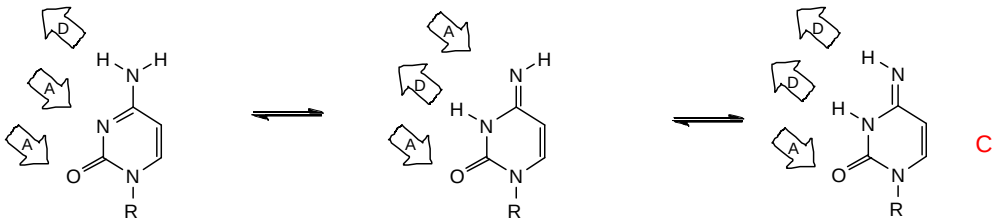

C
(=U)

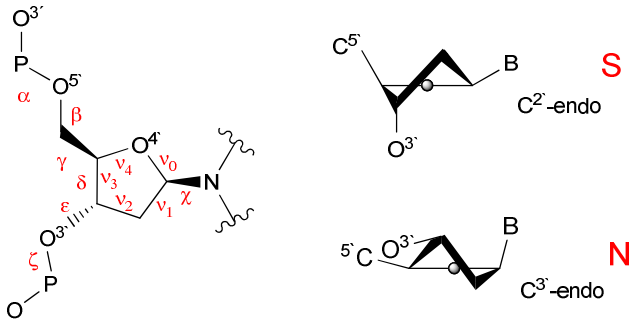
**Fig. 3** Keto-enol and amine-imine tautomerism in nucleoside bases (arrows denoted A and D symbolize acceptor and donor site, respectively).

Since this kind of metamorphosis of hydrogen bond acceptor and donor site would be problematic for a self-replicating system, this tautomerism has been intensively studied by UV, IR, NMR, crystallographic,[18,17] and quantum chemical methods.[19] The results can be summarized in the following way: the main tautomeric forms of the naturally occurring bases are the amino and the keto configuration (**Fig. 2)**.

### 1.2.2.3 Sugar-phosphate chain conformation

The conformational structure of a nucleotide within a nucleic acid is defined by the torsion angles $\alpha$, $\beta$, $\gamma$, $\delta$, $\varepsilon$, $\zeta$ in the phosphate backbone, $\nu_0$ to $\nu_4$ in the furanose ring, and $\chi$ for the glycosylic bond (**Fig.4**). Many of these torsion angles are interdependent, resulting in a simpler description of the nucleotides, defined by four parameters: the sugar pucker, the *syn-anti* conformation of the glycosylic bond, the orientation of $C^{4'}$-$C^{5'}$, and the shape of the phosphate ester bonds.

The puckering of the furanose ring is described by the major displacement of C2' and C3' from the plane defined through C1'-O4'-C4'. If the *endo* displacement of C2' is greater than the *exo* displacement of C3', the conformation is called C2'-*endo* and so on (the *endo* face of the furanose is on the same side as C5' and the nucleobase; the *exo* face is on the opposite side to the base) (**Fig.4, right**). Because these sugar puckers are located in the north (**N**) and south (**S**) domains of the pseudorotation cycle of the furanose ring, C2'-*endo* is also called **S**(outh) and C3'-*endo* **N**(orth). The energy barrier of the **N** and **S** conformation is less than 20 kJ/mol, resulting in a rapid equilibrium in solution, which follows the path of pseudorotation with O4'-*endo* as the transition state. [20]




| Torsion angle | Atoms involved |
|---|---|
| $\alpha$ | $_{(n-1)}$O3'-P-O5'-C5' |
| $\beta$ | P-O5'-C5'-C4' |
| $\gamma$ | O5'-C5'-C4'-C3' |
| $\delta$ | C5'-C4'-C3'-O3' |
| $\varepsilon$ | C4'-C3'-O3'-P |
| $\zeta$ | C3'-O3'-P-O5'$_{(n+1)}$ |
| $\chi$ | O4'-C1'-N1-C2 (pyrimidines)<br>O4'-C1'-N9-C4 (purines) |
| $\nu_0$ | C4'-O4'-C1'-C2' |
| $\nu_1$ | O4'-C1'-C2'-C3' |
| $\nu_2$ | C1'-C2'-C3'-C4' |
| $\nu_3$ | C2'-C3'-C4'-O4' |
| $\nu_4$ | C3'-C4'-O4'-C1' |

**Fig.4** Notation of torsion angle (according to IUPAC) for polynucleotide chains[20] and structures for the C2'-endo (S) and C3'-endo (N) preferred sugar puckers.

The plane of the nucleobase is almost perpendicular to that of the sugar. The bases may occupy two principal orientations. The *anti* conformer has the smaller H-6 (pyrimidine) or H-8 (purine) atom above the sugar ring, while the *syn* conformer has the larger O-2 (pyrimidine) or N-3 (purine) in that position. For pyrimidines it is only possible to occupy a narrow range of *anti* conformations while purines are found in a wider range of *anti* conformations which can even be

extended into the high-*anti* range (for 8-azapurines such as formycin)[21] (**Fig. 5**). One exception to the general preference for *anti* conformation is reported: guanine prefers the *syn* glycoside in mononucleotides, in alternating oligomers like dRib[$C_pG_pC_pG$], and in Z-DNA.[22]

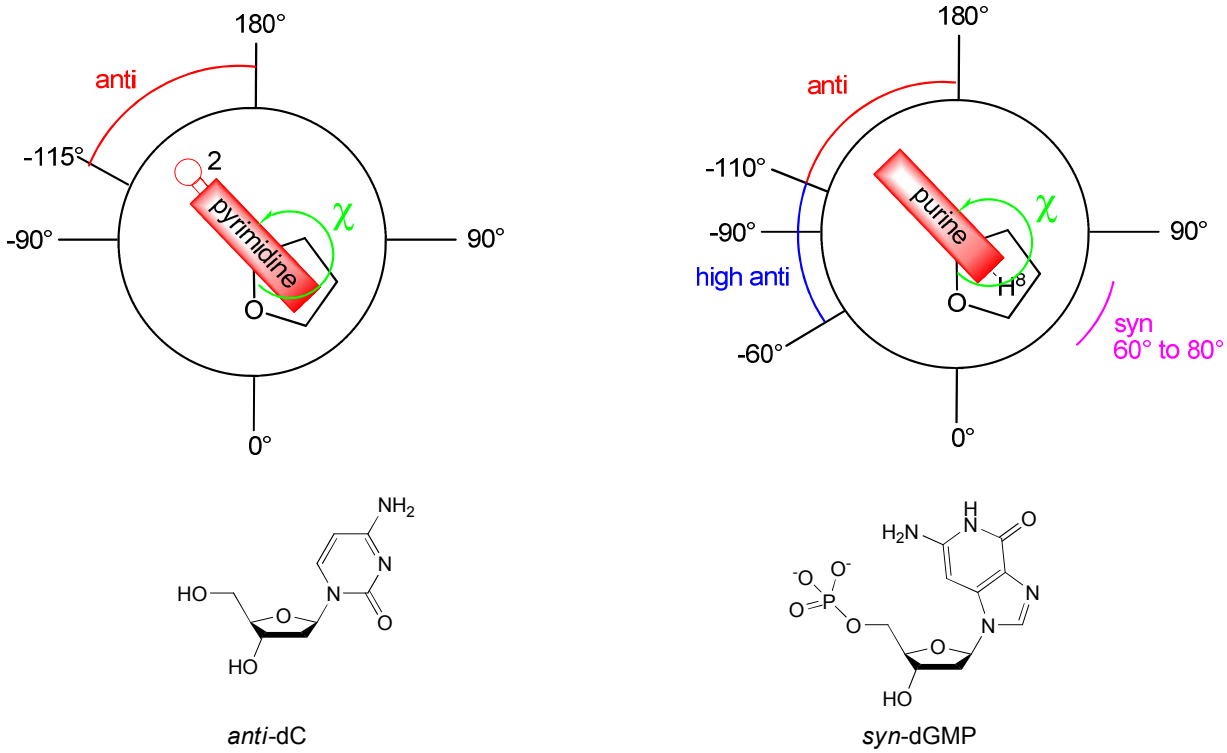



**Fig. 5** *Anti* and *syn* conformational ranges for glycosylic bonds ($\chi$) in pyrimidine (left) and purine (right) nucleosides and *anti* conformation of deoxycytidine (lower left) and *syn* conformation of deoxyguanosine-5'-phosphate (lower right).[23]

For the C4'-C5' bond, the favoured conformers are the **synclinal** (**sc**) and **antiperiplanar** (**ap**) rotamers. For pyrimidine nucleosides, **+sc** is preferred, while, for purine nucleosides, **+sc** and **ap** are equally populated. However, in the nucleotides, the 5'-phosphate reduces the conformational freedom and the dominant conformer is **+sc** (for γ) (**Fig. 6**). For the *syn* guanine, a value in the **ap** region was found.[22]

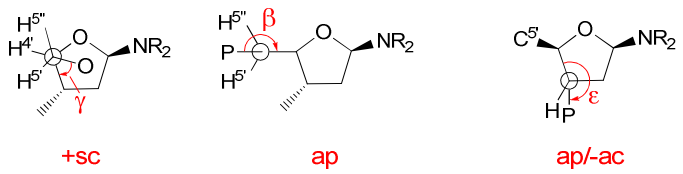



**Fig. 6** Preferred nucleotide conformations: +sc for C4'-C5' (left); ap for C5'-O5' (centre); and ap/-ac for C3'-O3' (right).

Phosphate diesters are tetrahedral at phosphorus and show **antiperiplanar** conformations for the C5'-O5' bond. Similarly, the C3'-O3' lies in the **antiperiplanar** to **anticlinal** range, resulting in the use of a virtual bond concept, in which the chains P5'-O5'-C5'-C4' and P3'-O3'-C3'-C4' can be analysed as rigid, planar units linked at phosphorus and at C4'.

### 1.2.2.4. Primary structure of DNA

Early work from *Klein* and *Thannhauser* established that the primary structure of DNA has each nucleoside joined by a phosphodiester from its 5'-hydroxyl group to the 3'-hydroxyl group of one neighbour and by a second phosphodiester from its 3'-hydroxyl group to the 5'-hydroxyl of its other neighbour.[24] There are no 5'-5' or 3'-3' linkages in regular DNA primary structure. This observation means that the uniqueness of a given DNA primary structure resides solely in the sequence of its bases.

### 1.2.2.5 Secondary structure of DNA

In 1953, *Watson* and *Crick* determined the structure of B-DNA by x-ray diffraction patterns (the counter ion being an alkali metal such as $Na^+$ and the relative humidity > 92%).[25] R. Dickerson determined, in 1980, the first X-ray crystal structure of a B-DNA (dGlc[CGCGAATTCGCG]) at near-atomic (1.9 Å) resolution.[26] If the relative humidity reduced

to 75%, B-DNA undergoes a reversible conformational change to the A-DNA. By solving the crystal structure of the self-complementary hexanucleotide dGlc[CGCGCG], Wang and Rich found a left-handed double helix (**Table 2**) in 1979, as the first reported example of Z-DNA.[27]

| | **A-DNA** | **B-DNA** | **Z-DNA** |
|---|---|---|---|
| Helical sense | Right-handed | Right-handed | Left-handed |
| Diameter | ~26 Å | ~20 Å | ~18 Å |
| Base pairs per helical turn | 11.6 | 10 | 12 (6 dimers) |
| Helical twist per base pair | 31° | 36° | 9° for pyrimidine-purine steps; 51° for purine-pyrimidine steps |
| Helix pitch (rise per turn) | 34 Å | 34 Å | 44 Å |
| Helix rise per base pair | 2.9 Å | 3.4 Å | 7.4 Å per dimer |
| Base tilt normal to the helix axis | 20° | 6° | 7° |
| Major groove | Narrow and deep | Wide and deep | Flat |
| Minor groove | Wide and shallow | Narrow and deep | Narrow and deep |
| Sugar pucker | C3'-*endo* | C2'-*endo* | C2'-*endo* for pyrimidines; C3'-*endo* for purines |
| Glycosidic bond | *Anti* | *Anti* | *Anti* for pyrimidines; *syn* for purines |

**Table 2** Structural features of ideal A-, B-, and Z-DNA.[28]

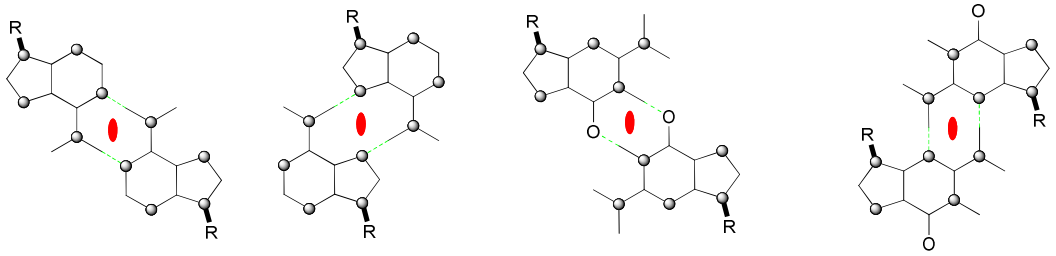
### 1.2.2.6 Hydrogen bonding motifs[29]

Under the assumption that at least two hydrogen bonds must form to produce a stable base pair, the four bases substituted at the glycosyl nitrogens (*N*1 In pyrimidine and *N*9 in purine) can be arranged in 28 different ways. The 28 base pairs are grouped in **Fig. 7** according to interactions between like and different bases in the purine-purine and pyrimidine-pyrimidine series, followed by purine-pyrimidine pairs. In each group, the orientation of the glycosyl *C*1'-*N* linkage can be

either unrelated by symmetry elements (asymmetric) or related by dyads (twofold axis) located perpendicular to or within the base-pair planes.

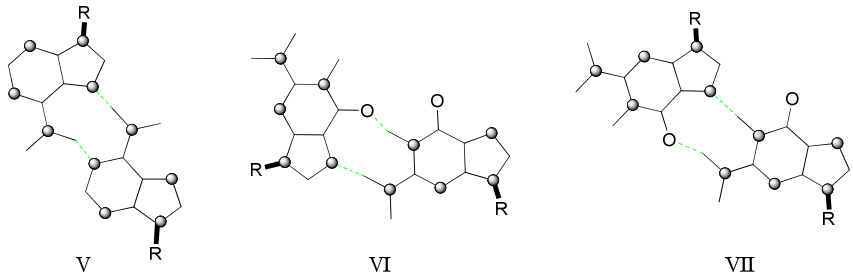
homo purine

symmetric




I II III IV

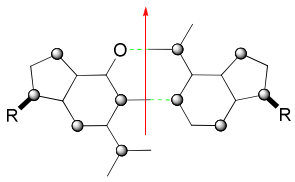
asymmetric




V VI VII

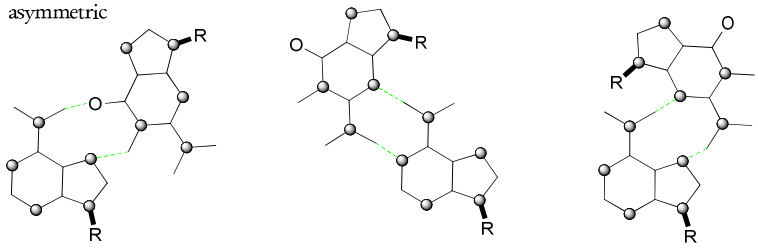
hetero purine

symmetric




VIII

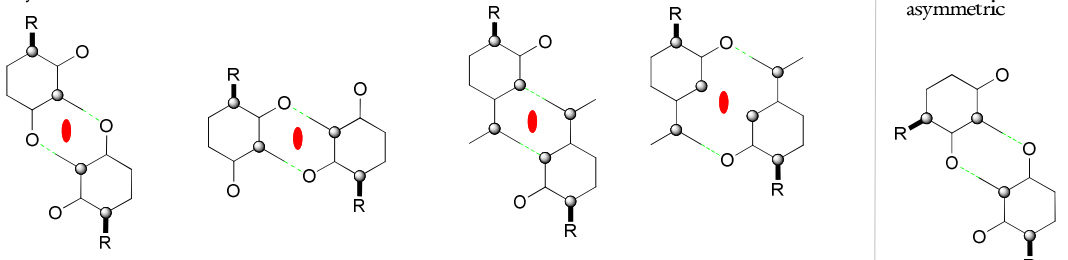
asymmetric




IX X XI

homo pyrimidine

symmetric




XII XIII XIV XV

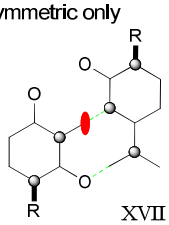
asymmetric



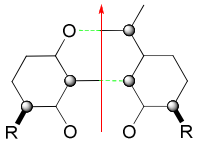

XVI

hetero pyrimidine

symmetric only



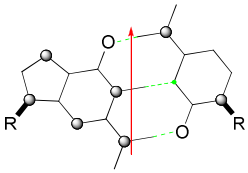
XVII



XVIII

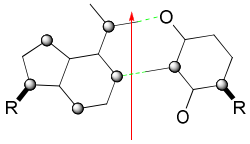
purine - pyrimidine

symmetric



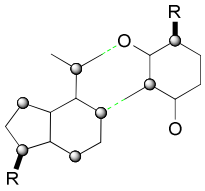
XIX

*Watson - Crick*

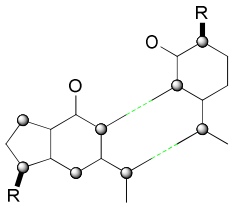


XX

asymmetric

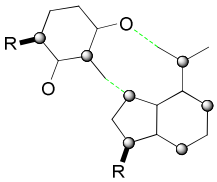


XXI



XXII

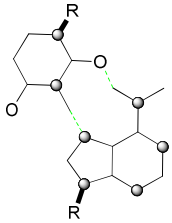
reversed
*Watson - Crick*



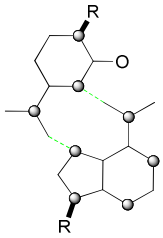
XXIII

*Hoogsteen*

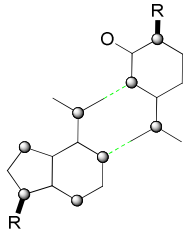
reversed
*Hoogsteen*



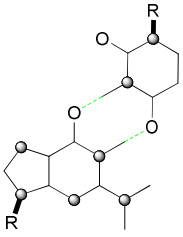
XXIV



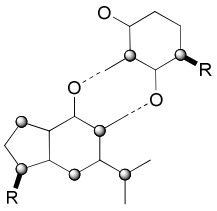
XXV



XXVI



XXVII



XXVIII

**Fig. 7** All 28 possible base pairs for A, G, U(T), and C involving at least two hydrogen bonds. Nitrogen atoms displayed as filled circles, glycosyl bonds as thick lines with R indicating ribose C1' atom. Symmetry elements for the orientation of the glycosyl C1'-N linkage  and  are twofold rotation axes vertical to and within the plane of the paper.[30]

In oligonucleotides, the symmetry elements are of special interest, because they relate not only the glycosidic bonds, but also the attached sugar-phosphate backbone.

# 2. Problem outline

A chemical rationalisation of the DNA structure should follow the methodology proposed by *Eschenmoser*:[31] two criteria should be taken into consideration: first, the relationship between the structure and its biological function; and second, the structure's potential for constitutional self-assembly.

Many papers have been published concerning the question why nature chooses ribose (and 2'-desoxyribose) as sugar backbones.[32] For the purine- and pyrimidinebases, they only refer to the possibility of their prebiotic origin.[31]

An open question is if other prebiotic accessible bases could also form base pairs with comparable strength and selectivity to the *Watson-Crick* base pairing in normal DNA. This question should be addressed in the same systematic way as the question why ribose and 2'-desoxyribose is selected for the backbone of RNA and DNA from all the praebiotic accessible sugars.

Today, it is known that cytidine is the least stable of the four nucleosides because of its tendency to undergo spontaneous deamination (hydrolysis) to uridine (t ½ = 340 yr at pH 7 and 25°).[33] With this experimental knowledge, a three-base codon derived from 2,4-diaminopyrimidine (D) as a single source and its hydrolysis products, cytosine (C) and uracil (U), was postulated: "By building a glycosidic bond through an exocyclic amino group a D nucleoside would be isosteric to A, complementary to U and a progenitor of C and U. By formation of the glycosidic bond in a C nucleoside by an exocyclic amine a fourth nucleoside (E) could be formed. E would be a harbinger of the modern G with a possible base pairing with C." (**Fig. 8**)[34]

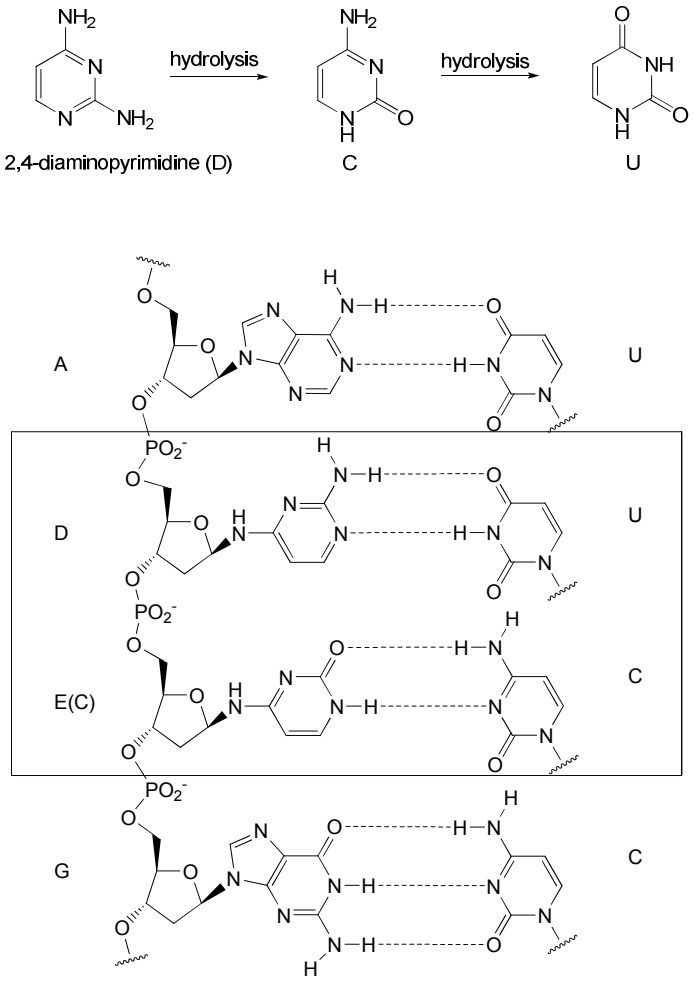
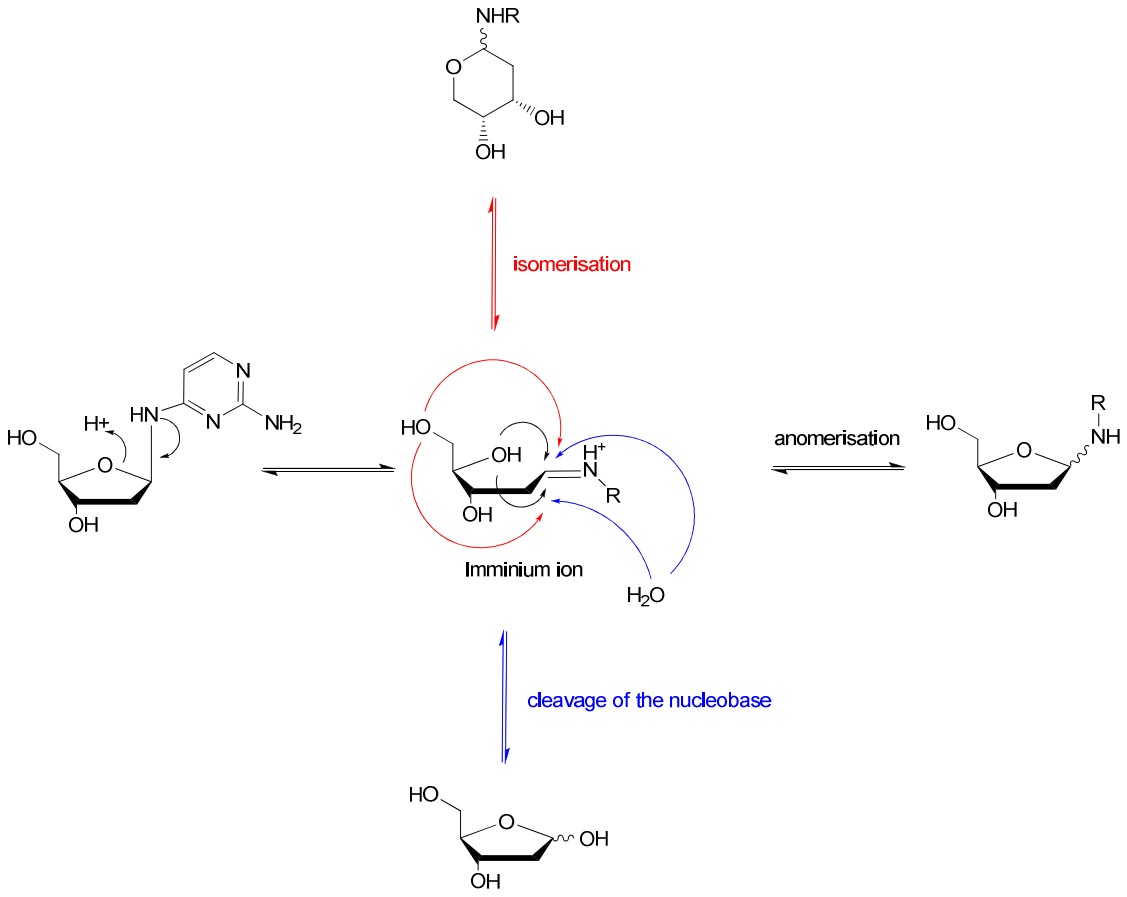



**Fig. 8** Hydrolysis cascade from diaminopyrimidine (D) to C to U (top) and pairing of D with U and E with C in a DNA system (bottom).

This proposal immediately leads to two questions:

1.) How stable are these exocyclic amino nucleosides (EANs) against the previously described anomerisation, isomerisation and cleavage of the nucleobase? [35]

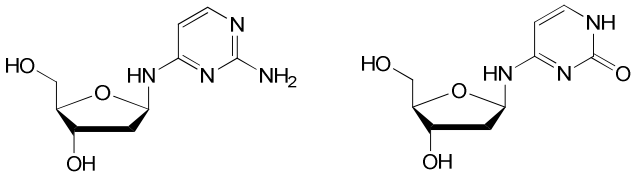



**Scheme 1** Possible mechanism of isomerisation (red arrows), anomerisation (black arrows) and cleavage of the nucleobase (blue arrows). [36]

2.) Are these EAN's capable of forming pseudo-*Watson-Crick* hydrogen bonds comparable in strength and selectivity with the ones formed by the naturally occurring nucleobases?

Many investigations have been conducted to determine stability parameters of EANs (rates of isomerisation, anomerisation, and cleavage of the nucleobase), in particular for FapydA and FapydG (**see 3.1.3.**). [37] Much less data is available in the literature concerning base-pairing properties of EANs, [38] due to their short lifetime, which requires calculations, the design of model systems or novel synthetic strategies.

The goal of this project was to design, prepare, and investigate a model system giving data for the base pairing properties of EANs and especially producing data on base-pairing properties of compounds **1** and **2** (**Fig. 9**).




**1** **2**

**Fig. 9** Exocyclic amino nucleosides of interest.

For the development the model system see chapter 4.1.

# 3. State of knowledge

## *3.1. Exocyclic amino nucleosides*

### 3.1.1. Biological significance of exocyclic amino nucleosides (EANs)

As a result of oxidative damage in natural DNA, EANs occur from purine nucleosides in the form of imidazole ring-opened formamidopyrimidines (fapydA derived from adenosine, fapydG derived from guanosine) and have been implicated in mutagenesis.[39] Clitocine (6-amino-5-nitro-4-($\beta$-D-ribofuranosylamino)pyrimidine), a secondary fungal metabolite, first isolated from the mushroom *Clitocybe inverse* in 1986 by *Kubo* et al.[40] exhibited strong insecticidal activity against the pink bollworm *Pectinophora gossypiella*. Later, it was found that clitocine has also strong cytostatic effect towards several leukaemia cell lines and is an inhibitor of adenosine kinase.[41] Synthetic EANs like 4-substituted 8-(D-ribofuranosylamino)pyrimido[5,4-*d*]pyrimidines show antitumor and antiviral properties (**Fig. 10**).[42]

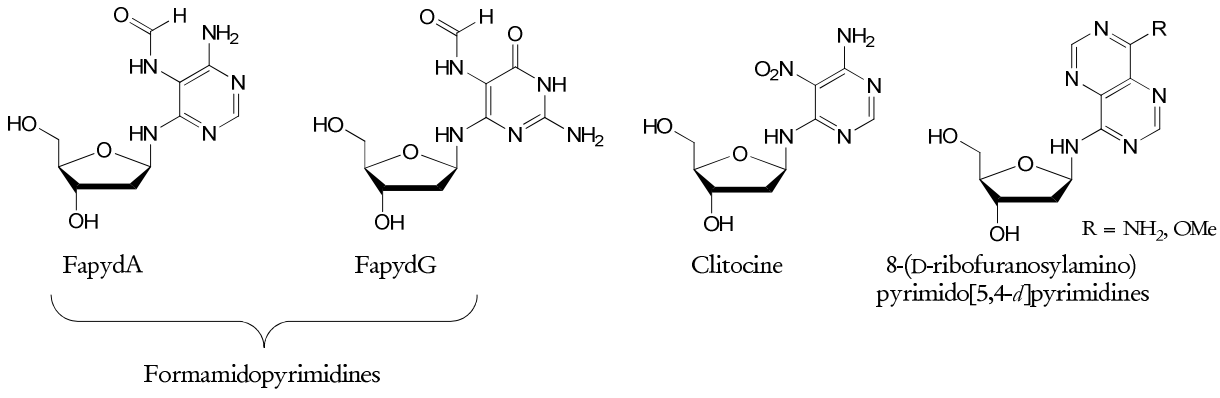



**Fig. 10** Examples of exocyclic amino nucleosides (EANs).

## 3.1.2. Hydrolysis (deamination) of 2,4-diaminopyrimidine and cytosine

The temperature-rate profile for the hydrolysis of cytosine to uracil was initially reported in 1972 by *Garret* and *Tsau*,[43] (**Fig. 11, left**) after *Shapiro* and *Klein* had found that the nucleobase cytosine and the nucleoside cytidin undergo a hydrolysis to uracil and uridine, respectively.[44] This deamination occurred under reaction conditions, which did not cause deamination in adenosine or guanosine. *Lindahl* and *Nyberg* discovered that the deamination rates of cytosine residues in single-stranded DNA, poly-d[C] and dCMP at pH 7.4 are comparable, though the cytosine residues in native double stranded DNA are deaminated at < 1% of the rate observed for dCMP or poly-d[C].[45] The hydrolysis of 2,4-diaminopyrimidine, studied in 1998 by *Levy* and *Miller* (**Fig. 11**, **right**), resulted in a mixture containing cytosine, isocytosine and uracil.[46]

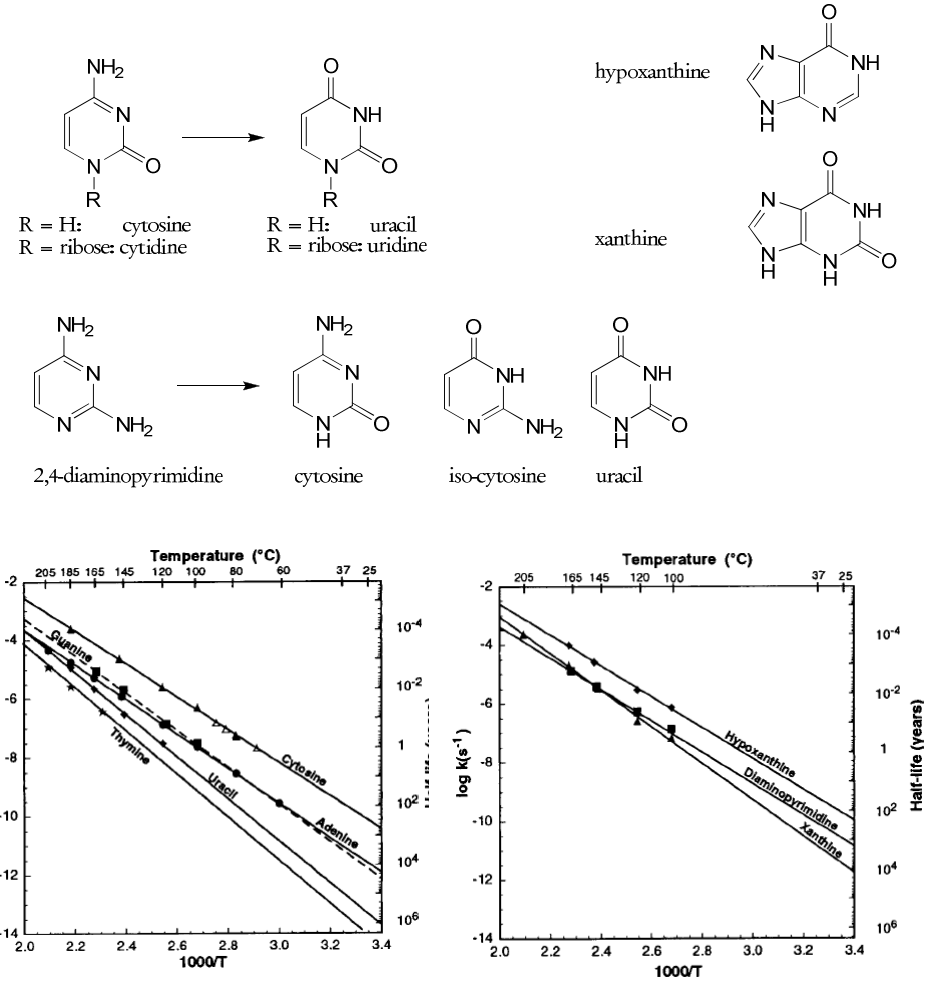


**Fig. 11** Observed hydrolysis processes (upper part) and *Arrhenius* plot for the decomposition of A, U, G, C, T (left) and xanthine, hypoxanthine, 2,4-diaminopyrimidine (right); (reported at pH 7.0).[46]

### 3.1.3. Available stability data for EANs concerning anomerisation, furanose-pyranose isomerisation, and cleavage of the nucleobase

Recent studies on triazine EANs performed by *Hysell* et al.[36] exhibited following effects: the stability of a triazine exocyclic amino nucleoside differ according to electron-deficiency of the nucleobase. **3** decomposes within 15 min in water, though **4** was stable in a $D_2O$-THF-d8 solution (**Fig. 12**).

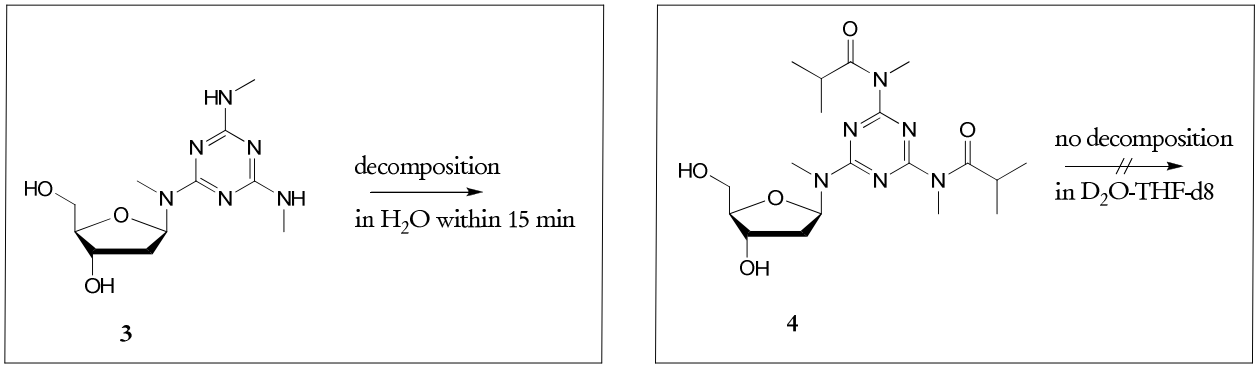



**Fig. 12** Examples of triazine EANs indicating the influence of electron-deficiency of the aromatic system on the stability in solution.

Further $^1$H-NMR studies with **5**$\beta$ demonstrated that the anomerisation to **5**$\beta$, **5**$\alpha$ occurred rapidly relative to the furanose-pyranose isomerisation (to **6**$\beta$, **6**$\alpha$). Nevertheless, the pyranose isomers **6**$\beta$, **6**$\alpha$ represent, as it was similarly reported for the formamidopyrimidines FapydA[35b] and FapydG,[35a] the thermodynamically favoured products. Relative to the anomerisation, the cleavage of the nucleobase is a slow process (**Fig. 13**).

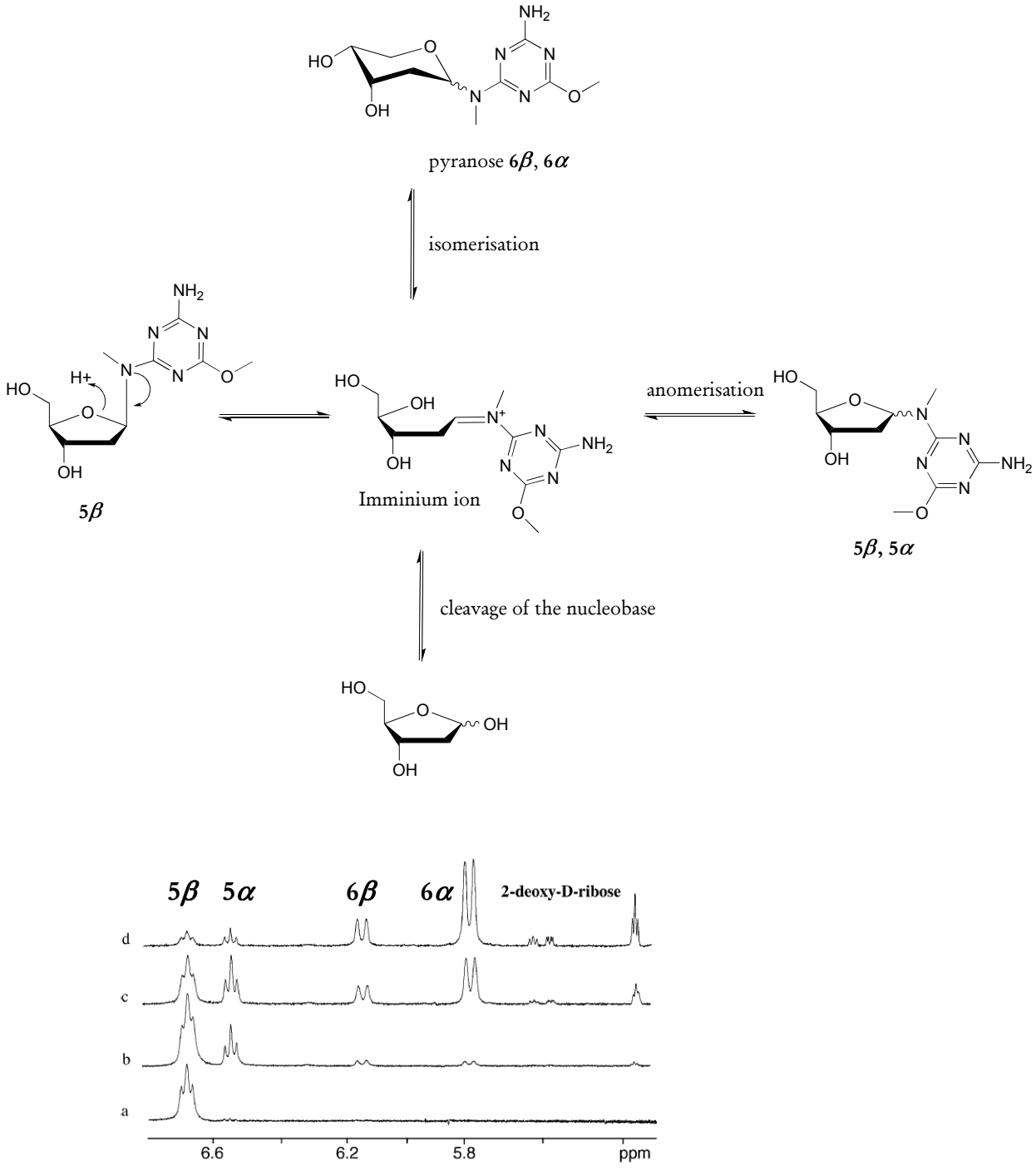



**Fig. 13** Anomerisation, isomerisation and cleavage of the nucleobase for the triazine EAN 5$\beta$ in $D_2O$, pH = 6.0, followed by $^1$H-NMR of the anomeric protons at (a) *t* = 0, (b) *t* = 1h, (c) *t* = 9h, (d) *t* = 30h.[36]

It was observed that the rate of isomerisation was increased at lower pH, as expected from the proposed mechanism: **5$\beta$** had a half-life of 10 h, 5 d, and more than 10 d at pH 6.0, 7.0, and

8.0, respectively. Although it was found that **5**$\beta$ was stable at pH = 11.0, the more electron-rich system **3** degraded within 15 min even at pH 11.0.

The isomerisation of **5**$\beta$ is slower in MeOH compared to $H_2O$; no isomerisation was observed for **5**$\beta$ in the aprotic solvents DMSO, acetonitrile, and acetone. The electron rich compound **3** also isomerised in aprotic polar solvents. Protection of the primary hydroxy at C5' of **3** as triisopropylsilylether resulted in a decrease of decomposition rate, explained by the loss of a possible intramolecular proton transfer or internal H-bonding assistance.

A recent study of the FapydG lesion was done by *Burgdorf* and *Carell*,[37b] studying EAN **7**$\beta$ as a model for the FapydG lesion (**Fig. 10, Fig. 14**). The authors point out that their results (half life for **7**$\beta$ of 37.8 h and 65.2 h for **7**$\alpha$, respectively in $CH_3CN/H_2O$ 1:1 at 50° C concerning the cleavage of the nucleobase) indicate a higher stability of the FapydG lesion than reported in an earlier publication by *Berger* and *Cadet*.[35a]

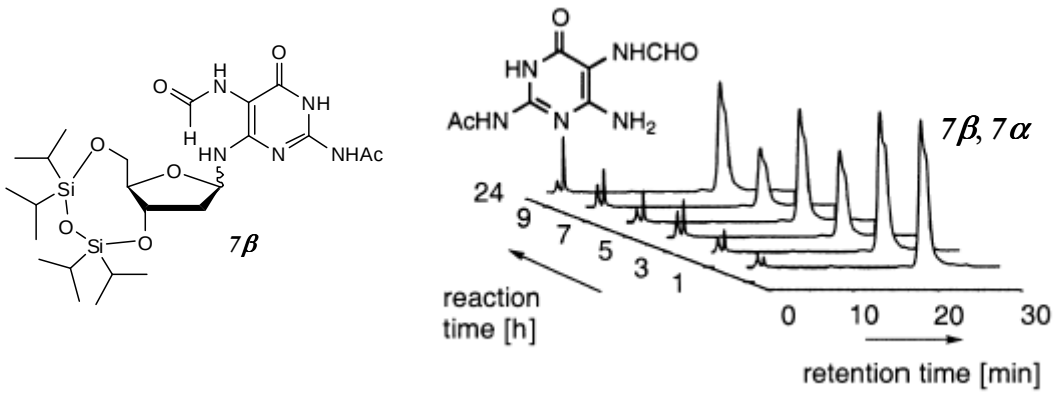



**Fig. 14** Model compound 7$\beta$ for the FapydG lesion (left) and HPLC-chromatogram of a 7$\beta$/7$\alpha$ 1:1 mixture in $CH_3CN/H_2O$ 1:1 at 50° C over 24 h (right).

The experimental observations from *Hysell* et al.,[36] indicate that rates for all decomposition processes (anomerisation, isomerisation and cleavage of the nucleobase) are dependent from four parameters:

1.) higher rates of decomposition in more electron-rich aromatic systems

2.) higher rates in protic solvents, compared to aprotic, polar solvents

3.) higher rates with unprotected hydroxy at C5'

4.) higher rates with decreasing pH.

A comparison of **7**$\beta$ with the FapydG lesion shows that all changes made in the model of *Burgdorf* et al. should result in a higher stability compared to FapydG: the acetyl protecting group results in a more electron-deficient system. The solvent used was $CH_3CN/H_2O$ 1:1 (compared to $H_2O$ used by *Berger* and *Cadet*) and the hydroxyl group at C5' was protected as a silylether. Therefore the higher stability from **7**$\beta$ compared to the results from *Berger* and *Cadet* may be explained by the results of *Hysell* et al.

### 3.1.4. Synthetic design for base pairing experiments of EANs.

For the synthesis of oligonucleotides containing EANs, it has to be considered that the isomerisation, anomerisation, and cleavage of the nucleobase (see. 3.1.3.) can be avoided. This topic has been addressed in three different approaches: the first attempt was described by *Greenberg* and coworkers for the fapydG[47] and fapydA[47b] lesion: *Greenberg* and coworkers applied a reverse $\beta$-cyanoethyl phosphoramidite (chain growth in the 5'→3' direction) with a dinucleotid-phosphoramidite as building block. This approach uses the fact that the instability of EANs is less pronounced in di- and oligonucleotides than mononucleosides and mononucleotides, allowing *Greenberg* and coworkers to introduce anomeric mixtures of fapydG and fapydA in an oligonucleotide chain (**Fig. 15**).

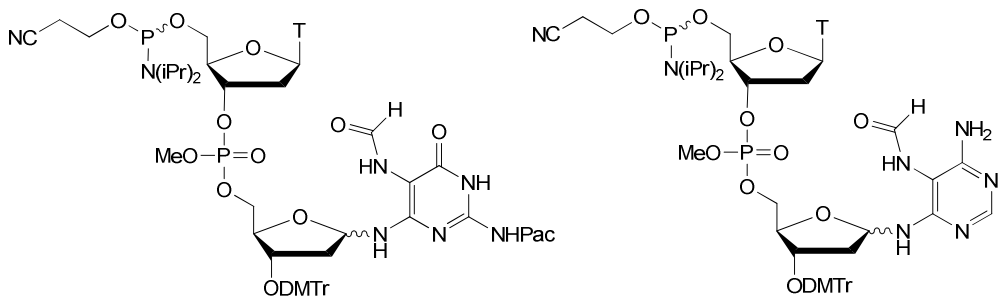


**Fig. 15** Dinucleotide of FapydG (left) and FapydA (right) for reversed 5'→3' oligonucleotide synthesis.

The second approach was also described by *Greenberg* and coworkers. This approach increases the stability of the glycosidic bond by an exchange of the exocyclic nitrogen, participating in the glycosidic bond by a carbon. By calculations[48] and experiments,[47b] it was shown that oligonucleotides containing *C*-nucleotides as model for the FapydG and FapydA lesion do not have significantly changed base pairing properties compared to natural *N*-nucleotides. This method allows for the selective insertion of a defined anomer by standard $\beta$-cyanoethyl phosphoramidite chemistry (chain growth in the 3'→5' direction) (**Fig. 16**).

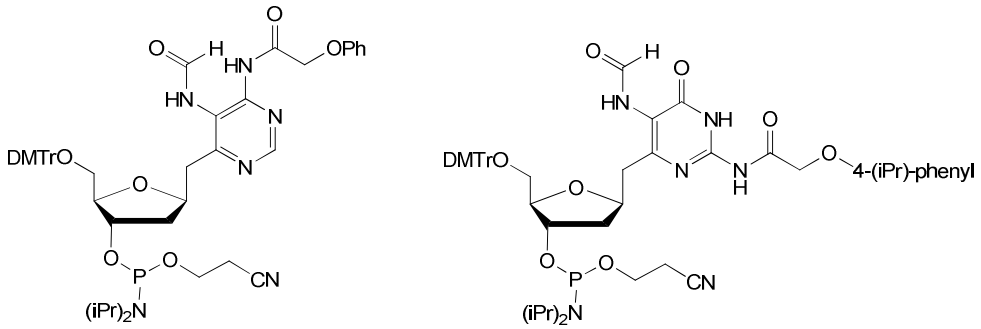


**Fig. 16** FapydA-*C*-phosphoramidite (left) and FapydG-*C*-phosphoramidite (right) for standard $\beta$-cyanoethyl phosphoramidite chemistry by *Greenberg* and coworkers.

The third approach, described by *Carell* and coworkers,[49,38a] is a variation of the second approach by *Greenberg* and coworkers described above: the glycosidic bond is strengthened by a

replacement of the oxygen in the furanose ring by a carbon, resulting in a replacement of the furanose against a cyclopentane. As already described for the second approach, this approach also allows for the selective insertion of a defined anomer by standard $\beta$-cyanoethyl phosphoramidite chemistry (chain growth in the 3'→5' direction). However, this approach has only been applied to the preparation of a model for the FapydG but not the FapydA lesion (**Fig. 17**).

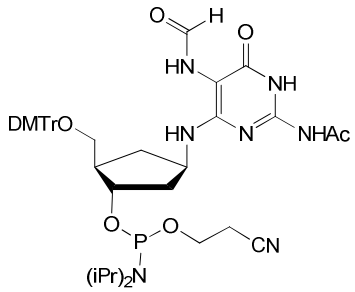



**Fig. 17** FapydG-cyclopentane-phosphoramidite as building block of a model compound for the FapydG lesion by *Carell* and coworkers.[38a, 49]

## *3.2 Known pyranose nucleic acid systems*

*Eschenmoser* and coworkers reported on the synthesis and characterisation of systems with pyranose-sugars and the regular purine- and pyrimidine-bases (adenine, guanine, thymine (uracil), cytosine).[50,32b] They reported that from the eight possible diastereomeric hexoses with (6'→4') connectivity (these systems have, in their backbone, six covalent bonds per monomer unit (as RNA and DNA)) neither the $\beta$-allo- (A) nor the $\beta$-altro- (B) nor the $\beta$-glucopyranosyl(6'→4')-system (C) shows base pairing properties comparable in strength and selectivity with the base pairing in RNA or DNA oligonucleotides (**Fig. 18**).

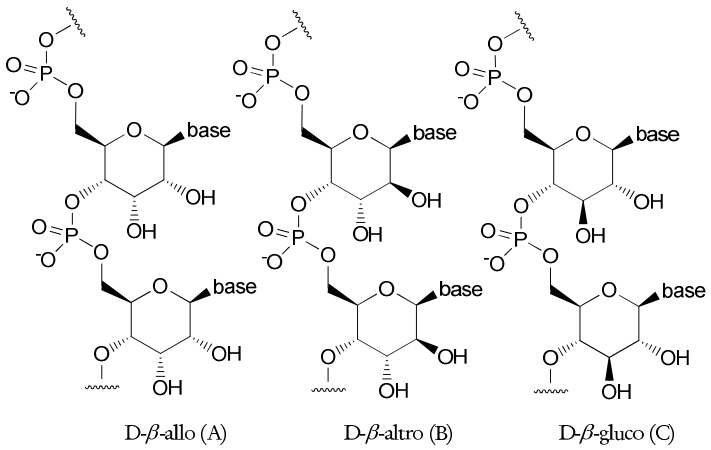



**Fig. 18** Members of the family of β-D-hexopyranosyl-(6'→4')-oligonucleotides.

In the unnatural 2',3'-dideoxy(6'→4')glucopyranosyl-system, a base pairing, which was stronger than the base pairing in DNA or RNA oligonucleotides, was found.[32c] The difference in base pairing strength was rationalised by steric hindrance between the equatorial 2'-OH group of a pyranosyl subunit and the neighbouring nucleobase. This hypothesis was tested by the synthesis of the 2'-deoxy-β-D-allopyranosyl (3'-OH, 2'-H) and the 3'-deoxy-β-D-allopyranosyl (2'-OH, 3'-H) systems. The former showed strong base pairing whereas the latter showed only weak base pairing (**Fig. 19**).[51,50,32b] Recent results from the first reported x-ray structure of a homo-DNA octamer duplex (ddGlc[CGAATTCG]$_2$) indicated that hydroxy groups attached to the 2',3'-dideoxyglucopyranose of several residues in the various configurations display short contacts to either atoms from adjacent base, sugars, or the phosphate group: *Egli* et al. point to overcrowding of the backbone as the most likely reason for the inability of fully hydroxylated hexopyranose-based nucleic acid analogues to form stable duplexes.[52]

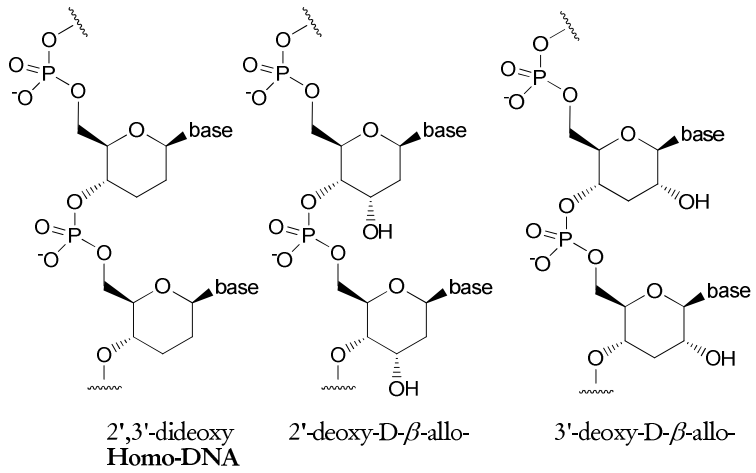



**Fig. 19** Pyranose oligonucleotides.

## *3.3 Base pairing in the 2',3'-dideoxy(6'→4')glucopyranosyl (homo-DNA)-system* [32c]

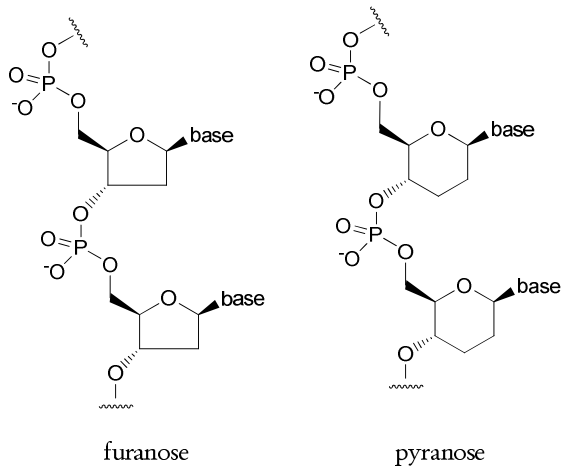



**Fig. 20** Constitution and configuration of homo-DNA oligonucleotides compared to DNA oligonucleotides.

In the homo-DNA system (**Fig. 20**), a different base pairing pattern (compared to DNA) is observed:

| oligonucleotide | $T_m$ [°C] | $\Delta H$ [kcal/mol] | $T * \Delta S$ (25 °C) [kcal/mol] | $\Delta G$ [kcal/mol] | Hyperchromicity (wavelength) [%] |
|---|---|---|---|---|---|
| ddGlc[$A_6$] | 47 (15 μM) | -39.4 | -30.2 | -9.2 | 7 (260 nm) |
| ddGlc[$(AT)_6$] | 59 (9.3 μM) | -60.2 | -47.1 | -13.1 | 25 (260 nm) |
| d[$(AT)_6$] | 24 (8.9 μM) | -65.2 | -59.7 | -5.5 | 35 (260 nm) |
| ddGlc[$G_6$] | 38 (20 μM) | -45.2 | -36.8 | -8.4 | 13 (250 nm) |
| ddGlc[$G_6$], ddGlc[$C_6$] | 61 (50 μM) | -39.5 | -28.6 | -10.9 | 22 (280 nm) |
| ddGlc[$(CA)_6$] | 29 (8.3 μM) | -53.0 | -45.1 | -7.9 | 12 (260 nm) |

**Table 3** Thermodynamic data of homo-DNA- (ddGlc) and DNA-oligonucleotides.[32c]

**Adenine pairs with adenine**. Different from the DNA hexamer dRib[$A_6$], the ddGlc[$A_6$] showed a duplex formation deducible from changes in UV- and CD-spectroscopy. Analogous to DNA-oligonucleotides the melting temperature ($T_m$) of ddGlc[$A_n$] (n =6, 10, 12) is dependant on the salt concentration and increases in a range from 0.015 to 1.5 M NaCl with increasing salt concentration (d$T_m$/dln(c)-pitch of +2°/M) but less pronounced than for DNA-oligonucleotides (+8°/M).[29] Increasing concentrations of urea decrease the $T_m$ value, similar to that seen for DNA oligonucleotides.

**Adenine pairs with thymine (but weaker than with adenine)**. The addition of ddGlc[$T_6$] did not influence the UV-melting curve of ddGlc[$A_6$]. The observation of A-T pairing resulted from alternating, self-complementary sequences ddGlc[$(AT)_n$] (n = 3, 4).

**Guanine pairs with guanine**. The hexamer ddGlc[$G_6$] shows intermolecular self-pairing properties similar to ddGlc[$A_6$].

**Guanine pairs with cytosine (stronger than with itself).** Different from the addition of ddGlc[$T_6$] to ddGlc[$A_6$] the self-pairing complex ddGlc[$G_6$] is broken up by the addition of ddGlc[$C_6$]. Phenomenologically, the ddGlc[purin]—ddGlc[purin] complexes can be differentiated from ddGlc[purin]—ddGlc[pyrimidine] pairing-complexes through different hyperchromicity ranges: UV-melting curves of ddGlc[purin]—ddGlc[purin] duplexes typically show

hyperchromicity values (at 260 nm for ddGlc[$A_n$] —ddGlc[$A_n$] and 250 nm for ddGlc[$G_n$]—ddGlc[$G_n$], respectively) of less than < 13%, during ddGlc[purin]—ddGlc[pyrimidine] duplexes possess hyperchromicity values of > 20% (at 260 nm for ddGlc[$(AT)_n$]—ddGlc[$(AT)_n$] and 280 nm for ddGlc[$G_n$]—ddGlc[$C_n$]).

**Adenine pairs with cytosine (but weaker than with thymine).** In ddGlc[$(AC)_n$], an oligonucleotide with sufficient chain length (n > 4), a duplex formation was observed, in contrast to the corresponding DNA oligonucleotide dRib[$(AC)_n$]. The melting temperatures of ddGlc[$(AC)_n$]-pairing complexes were, at the same concentration, about 20-30 °C lower than the corresponding ddGlc[$(AT)_n$] complexes, but still higher than the corresponding DNA oligonucleotides dRib[$(AT)_n$].

DdGlc[purine]-ddGlc[pyrimidine] and ddGlc[purine]-ddGlc[purine] prefer antiparallel strand orientation. Purine-pyrimidine paired duplexes of the homo-DNA series are thermodynamically more stabile than the corresponding duplexes in the DNA series, due to entropic (and not enthalpic) reasons (**Table 3**).

Homo-DNA sequences do not cross-pair with complementary sequences containing DNA-, RNA- nor any other oligonucleotide analogue backbone that has been identified to date.[53]

Recent results from the first reported X-ray structure of a homo-DNA oligonucleotide showed that the backbone-base inclination (**Fig. 21**) determines the relative polarity of paired strands in duplexes (antiparallel versus parallel) and the potential for cross-pairing between different nucleic acid systems:[52] Nucleic acid-pairing systems with significant positive or negative inclinations are essentially unable to form parallel arrangements involving two or more oligonucleotides and reverse *Watson-Crick* base pairs. A difference of 20° or more in the backbone-base inclination exhibited by two oligonucleotide systems would render cross pairing impossible.

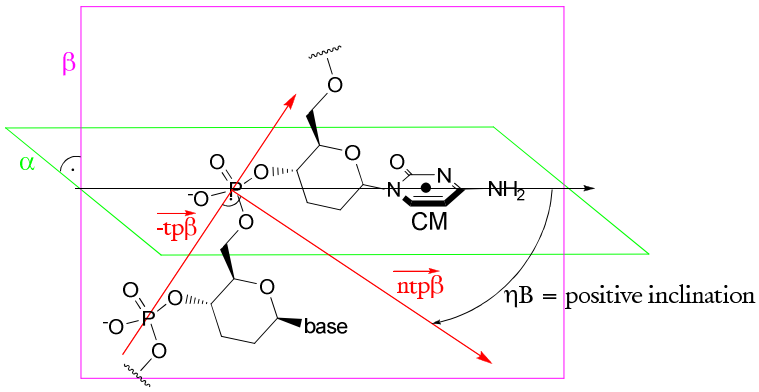



**Fig. 21** Backbone-base inclination: ηB defined as angle between vectors P-CM and ntpβ with (α) best plane through the $base_n$, (β) plane normal to α containing P and CM (base's centre of mass), (tpβ) orthogonal projection on plane  of vector P-$P_{n+1}$ at position P, (ntpβ) vector orthogonal to tpβ in plane β with P as origin.[52]

In all following parings all base conformations are *anti*. The adenine-adenine pairing in the homo-DNA series occurs according to the reverse *Hoogsteen*-mode with an antiparallel strand orientation. The adenine-thymine pairing in homo-DNA oligonucleotides is formed in the *Watson-Crick* mode with an antiparallel strand orientation. The guanine-guanine pairing in homo-DNA prefers antiparallel strand orientation. The guanine-cytosine pairing in homo-DNA prefers a *Watson-Crick* mode (as the A-T pairing) with antiparallel strand orientation. The constitution of the adenine-cytosine has not been proven, but experimental results indicate a reverse *Hoogsteen* pairing (**Fig. 22**): A decrease of melting-temperature with decreasing pH and a complete loss of pairing by replacement of the adenine by 7-*C*-adenine was observed. This experimental results excluded a *Watson-Crick* pairing of a protonated adenine with cytosine (would be stronger with lower pH) and a reversed *Watson-Crick* (*N*7 is not involved in pairing, replacement of *N*7 with *C* would not influence base pairing)).

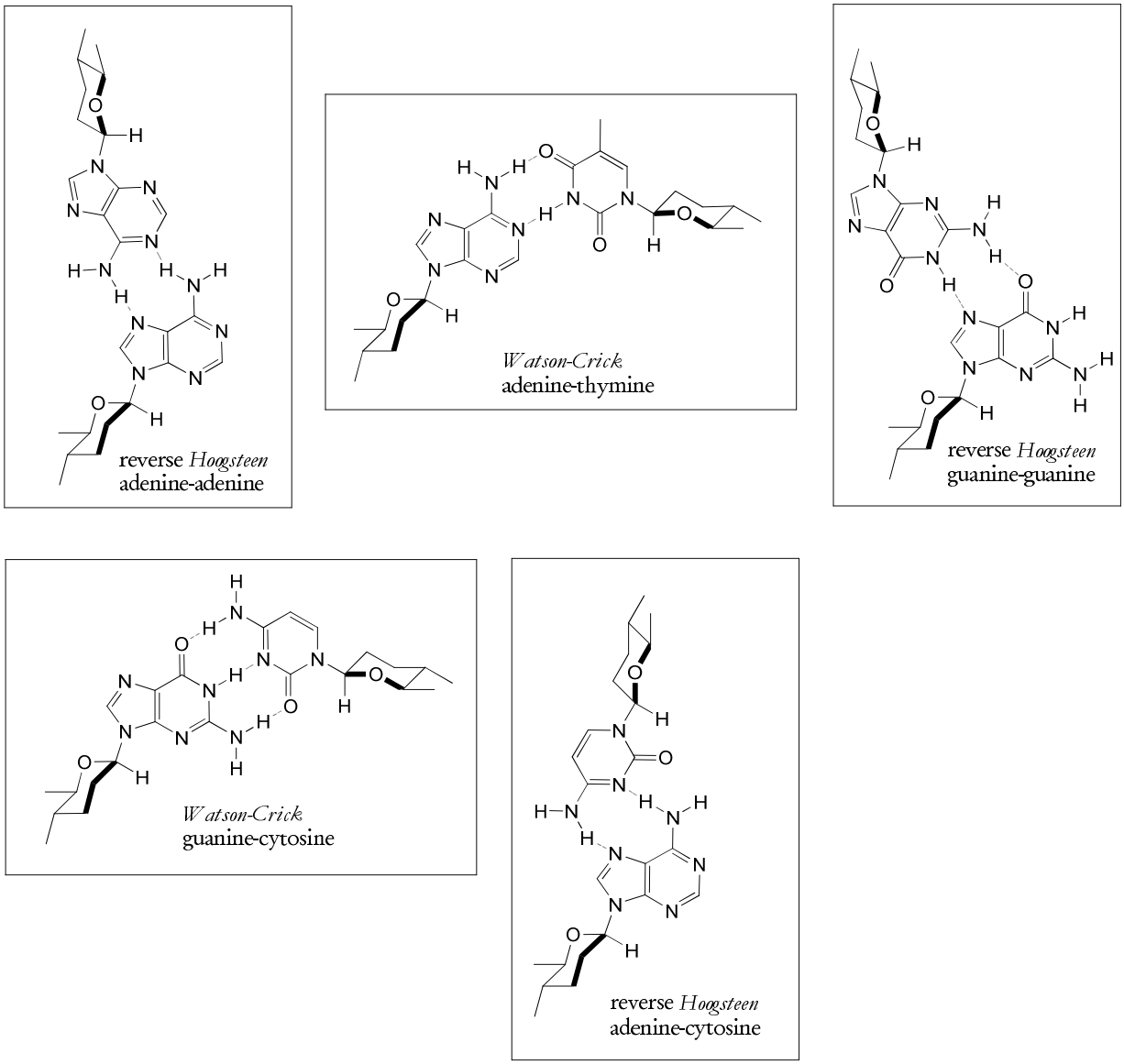
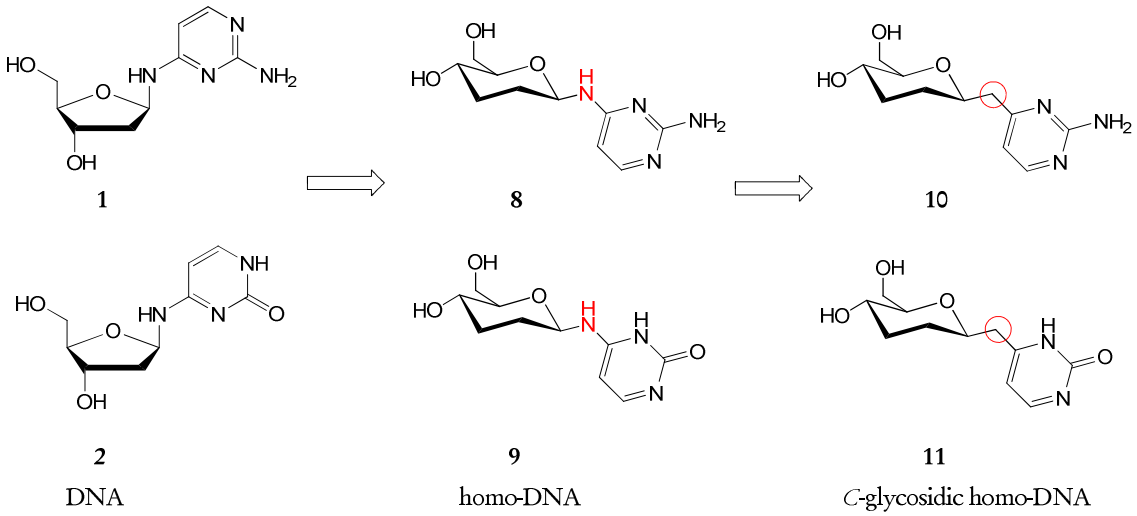



**Fig. 22** Constitution of homo-DNA-pairing (from upper left to bottom right): adenine-adenine, adenine-thymine, guanine-guanine, guanine-cytosine and adenine-cytosine.

# 4. Own work

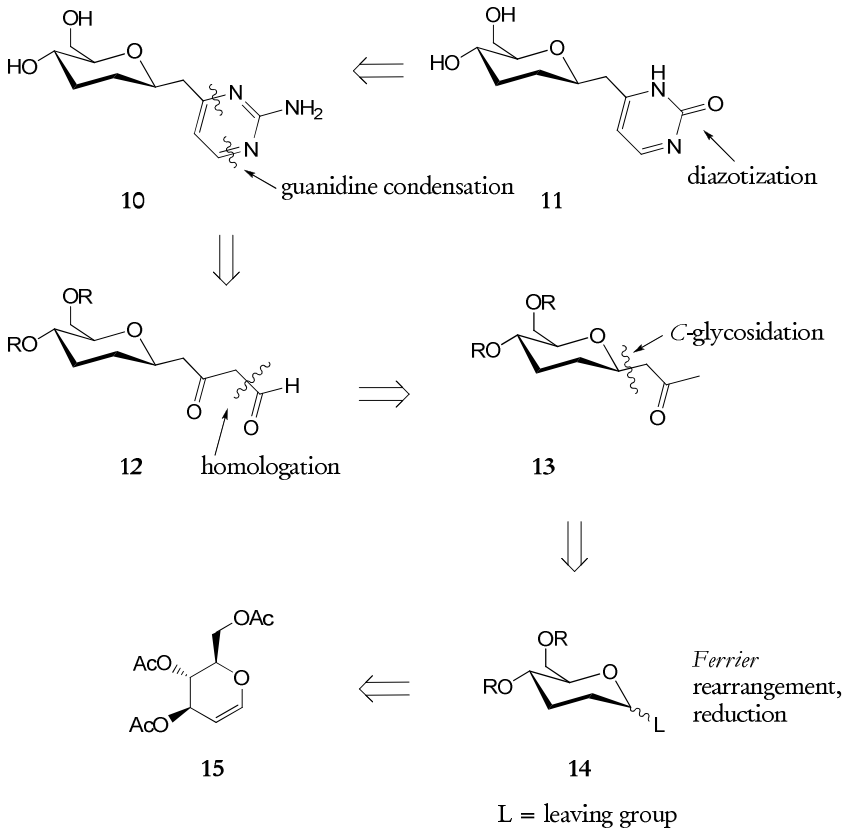
## *4.1. Development of a model system*

EANs undergo a furanose-pyranose isomerisation in which the pyranose isomers are the thermodynamically favored species for triazine EANs[36] as well as for diaminopyrimidine EANs[35] (see 3.1.3). This observation led us to choose a pyranose nucleic acid analogue, 2',3'-dideoxy(6'→4')glucopyranosyl (homo-DNA), as a model system for base pairing studies (**Scheme 2**). For a stabilization of the instable glycosidic bond in the EAN (anomerisation, isomerisation and cleavage of the nucleobase) an approach was applied, first reported by *Greenberg* and coworkers.[47b] to study the pyrimidine EANs, FapydA and FapydG, replacing the exocyclic nitrogen by a carbon (**Scheme 2**), allowing the study of the base pairing properties of a stable species in the absence of an isomerisation- and anomerisation equilibrium.




**Scheme 2** Development of a model system for base pairing studies.

## *4.2. Retrosynthetic analysis*




**Scheme 3** Retrosynthetic analysis of nucleosides **10** and **11**.

**10** and **11** represent the products of condensation of guanidine and urea with *β*-ketoaldehyde **12**, or a *β*-ketoaldehyde equivalent, respectively (**Scheme 3**). Due to the poor nucleophilicity of urea (→problems for the condensation), it should be possible to prepare 2-oxopyrimidine-nucleoside **11** from **10** by activation of the amino group as a diazonium ion followed by hydrolysis.

In *β*-ketoaldehyde **12,** the protecting groups R have to be chosen to survive basic conditions, which are necessary for the condensation, making ester protecting groups (for example acetyl- or benzoyl-protecting groups) unsuitable for this purpose.

*β*-ketoaldehyde **12** should be prepared in an *aldol*-type reaction from ketone **13**. In the key step of the synthesis, ketone **13** has to be prepared diastereoselectively as the *β*-anomer from

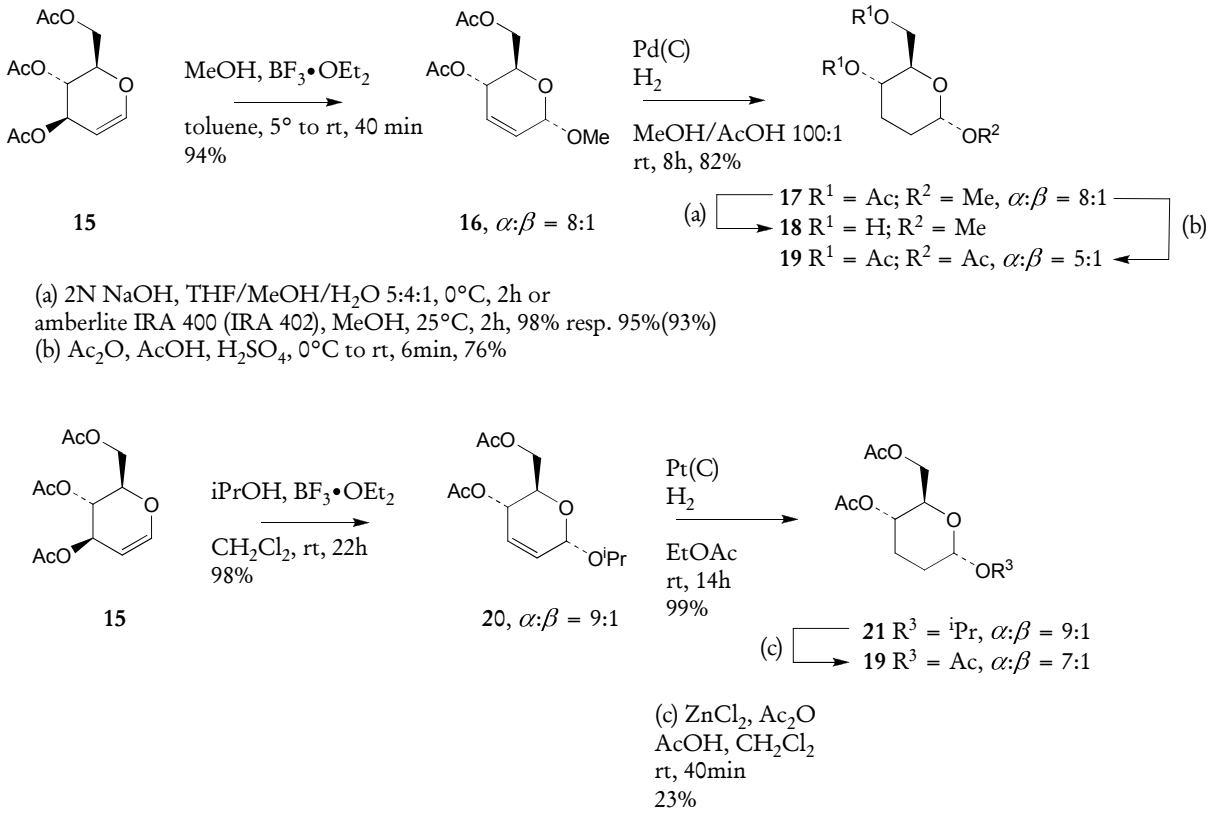
glycoside **14**. Because it was reported from *Eschenmoser* and coworkers for the *Vorbrüggen*-nucleoside synthesis of a non *C*(1) activated pyranoside,[32a] and recently also for *C*-glycosylations of pyranoses in *C*(1) activated glycosides by *Woerpel* and coworkers,[54] that the configuration at *C*(1) did not affect the diastereoselectivity of the nucleosidation and *C*-glycosylation, in either case, we planed the synthesis of glycoside **14** as an anomeric mixture.

Glycoside **14** should be prepared from 3,4,6-tris-*O*-acetyl-D-glucal by a *Ferrier* rearrangement, resulting in a 2,3-enopyranoside, from which **14**, as precursor in the *C*-glycosylation, should be obtained after hydrogenolytic reduction of the double bond and protecting group manipulations.

## *4.3. Synthesis*

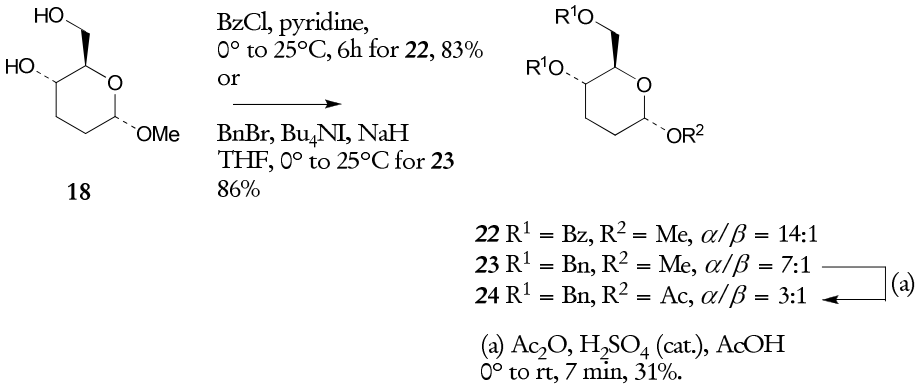
### 4.3.1 Synthesis of *C*-glycosides

3,4,6-tris-*O*-acetyl-D-glucal **15** was converted by a *Ferrier* rearrangement,[55,32a] to the methyl-4,6-di-*O*-acetyl-$\alpha/\beta$-D-erythro-hex-2-enopyranoside **16** with a diastereomeric ratio **16**$\alpha$/**16**$\beta$ = 8:1 (according to integration of GC and $^1$H-NMR data) in 94% yield (**Scheme 4**). The following hydrogenation with Pd(C) in acidic MeOH resulted in saturated pyranoside **17** (**17**$\alpha$/**17**$\beta$ = 8:1, GC, $^1$H-NMR) in over 80% yield. Basic hydrolysis of 4,6-bisacetate **17** with 2 N NaOH in THF/MeOH/$H_2O$ 5:4:1 at 0 °C, or with the strongly basic anion exchange resin IRA 400 (or IRA 402) in MeOH, resulted in pyranoside-4,6-diol **18** in 98% (2 N NaOH), 95% (IRA 400), and 93% (IRA 402) yields, respectively. C(1) activation under acidic conditions with AcOH, $H_2SO_4$ gave access to the 1,4,6-tris-*O*-acetylpyranoside **19** in 76% yield (**19**$\alpha$/**19**$\beta$ = 5:1, GC).[56]




**Scheme 4** Synthesis of glycosides **17-19**, and **21** from 3,4,6-tris-*O*-acetyl-D-glucal according to *Ferrier* (top) and *Isobe* (bottom), respectively.

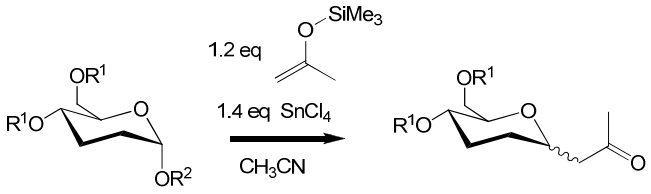
A slightly different strategy for the synthesis of 1,4,6-trisacetate **19** from 3,4,6-tris-*O*-acetyl-D-glucal by *Jiang* and *Isobe*,[57] also starting with a *Ferrier* rearrangement, yielded hex-2-eno-pyranoside **20** (**20**$\alpha$/**20**$\beta$ = 9:1 (GC)) in over 90% yield. Hydrogenation to the fully saturated 4,6-bisacetate with Pt(C) yielded in over 95% as $\alpha$-D/$\beta$-D = 9:1 anomeric mixture (GC, $^1$H-NMR) pyranoside **21**. The following C(1) activation the 1,4,6-trisacetate **19** under *Lewis* acidic assistance with $ZnCl_2$ proceeded in only 23% yield ($\alpha$-D/$\beta$-D = 7:1 (GC)) with the *C*(1)-OH pyranoside as side product, making the procedure of *Ferrier* and *Prassad* superior for the preparation of *C*(1) activated pyranoside **19**.




**Scheme 5** Synthesis of 4,6-bis-*O*-benzoyl-pyranoside **22** and 4,6-bis-*O*-benzylpyranoside **23** and 1-*O*Ac-4,6-bis-*O*-benzyl-pyranoside **24.**

Esterification with benzoyl chloride in pyridine resulted in bis-*O*-benzoylpyranoside **22** (yield 83%, **22**$\alpha$/**22**$\beta$ = 14:1 (GC, $^1$H-NMR). Bis-*O*-benzylpyranoside **23** was obtained from **18** in THF with benzyl bromide (yield 86%, **23**$\alpha$/**23**$\beta$ = 7:1 (GC)) (**Scheme 5**). The synthesis of 1-*O*Ac-4,6-bis-*O*-benzyl-pyranoside **24** from 1-*O*Me-pyranoside **23**, under similar reaction conditions already applied in the preparation of 1,4,6-trisacetate **19**, could not be optimized to a yield better than 31%; increasing the reaction time resulted in cleavage of the benzyl protecting groups under the strongly acidic reaction conditions (AcOH, $H_2SO_4$ (cat.)). A decrease in the reaction time resulted in recovery of starting material. All attempts to activate C(1) in pyranoside **23** as $\alpha$-halogenoether ($\alpha$-chloro- and $\alpha$-bromoether) failed (results not shown).

The silylenolether of acetone was prepared from acetone, $Me_3SiCl$ and NaI in a procedure described by *Cazeau* et al.[58] Under the coupling conditions to form *C*-glycosides (1.2 eq silylenolether of acetone, 1.4 eq distilled $SnCl_4$ (bulb-to-bulb-distillation), dry $CH_3CN$), the C(1) activated pyranoside **19** resulted in the generation of undesired $\alpha$-*C*-glycoside **25**$\alpha$ (**25**$\alpha$/**25**$\beta$ = 66/34, $^1$H-NMR) as the major anomer (yield 78%) (**Table 4**).



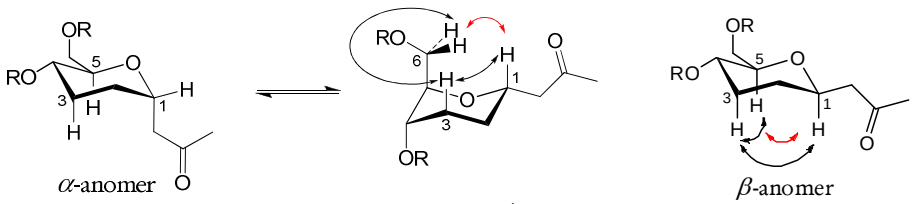

**17**
**19**
**22**
**23**

**25**
**26**
**27**

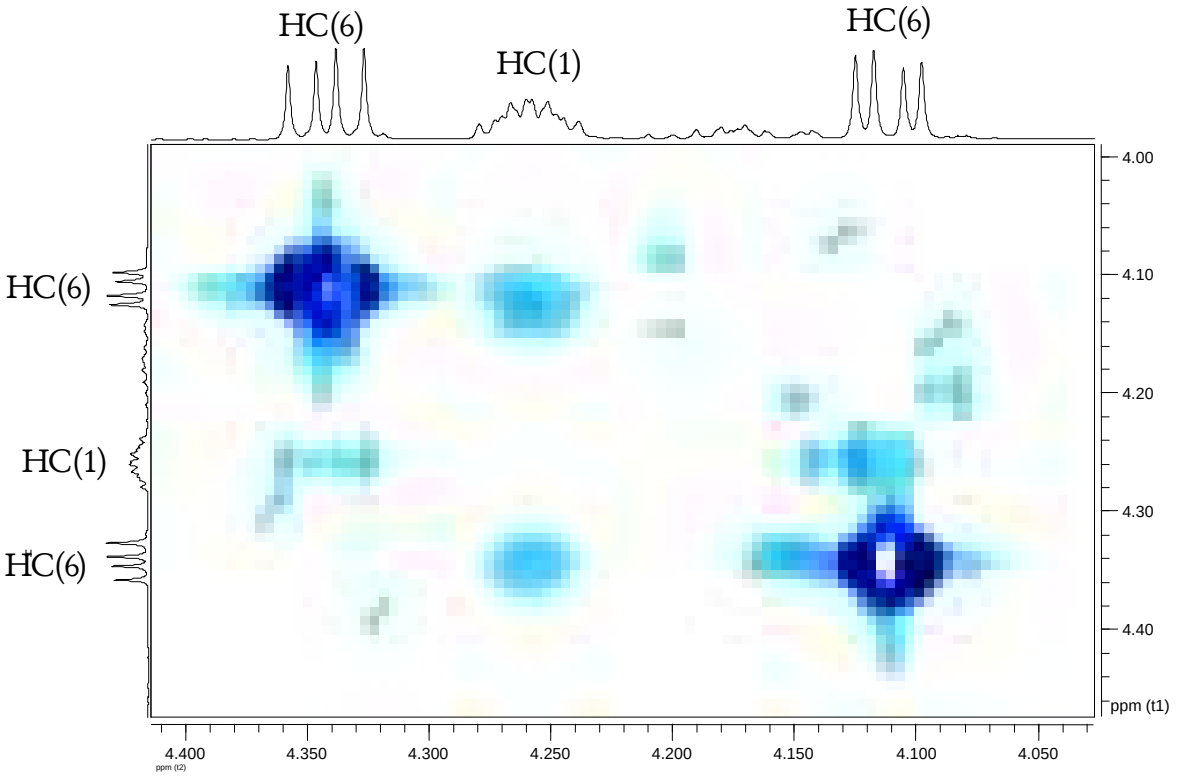
| Substrate | Product | $R^1$ | $R^2$ | Temp. [°C] | Additive | $\alpha/\beta$ | Yield | Reaction time |
|---|---|---|---|---|---|---|---|---|
| **17** | **25** | Ac | Me | 25 | - | 47/53 | 44% | 6 h |
| **17** | **25** | Ac | Me | 25 | 3 Å MS | 71/29 | 53% | 6 h |
| **19** | **25** | Ac | Ac | -78 | - | 66/34 | 78% | 2 h |
| **22** | **26** | Bz | Me | 25 | - | 69/31 | 76% | 6 h |
| **22** | **26** | Bz | Me | 25 | 3 Å MS | 77/23 | 49% | 6 h |
| **23** | **27** | Bn | Me | 25 | - | 18/82 | 51% | 6 h |
| **23** | **27** | Bn | Me | 25 | 3 Å MS | 69/31 | 63% | 6 h |
| **23** | **27** | Bn | Me | 25 | - | 12/88 | 17% | 21 h |

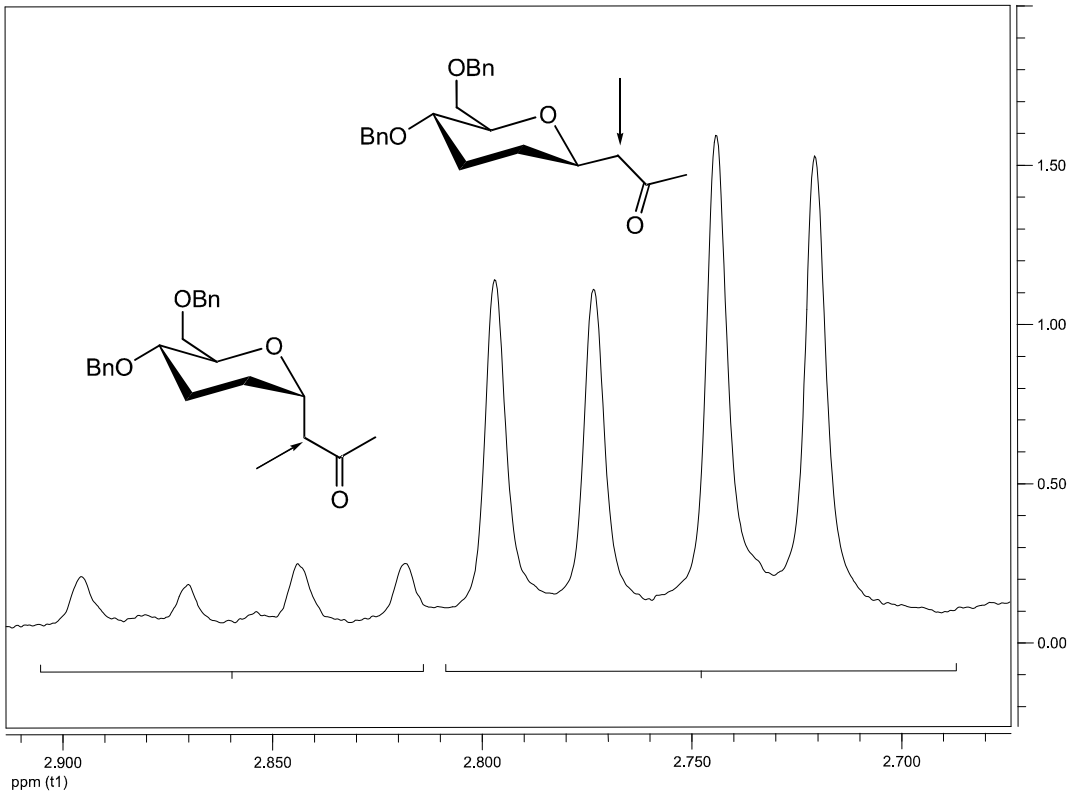
**Table 4** Formation of *C*-glycosides **25-27**.

**25**$\alpha$ and **25**$\beta$ were separated by preparative column chromatography ($SiO_2$, $Et_2O$/hexane 3:1) and the structure was assigned by COSY and NOESY experiments: all expected 1,3-diaxial NOEs in the separated anomers **25**$\alpha$ and **25**$\beta$ were observed. In **25**$\beta$ NOEs between HC(1) and $H_{ax.}C(3)$, $H_{ax.}C(3)$ and HC(5) and more importantly between HC(1) and HC(5) (an NOE not detected for **25**$\alpha$) were observed (**Fig. 23**, upper right). In **25**$\alpha$ NOEs resulting from an inverted chair conformation were observed between HC(1) and 1 HC(3), 1 HC(3) and both $H_2C(6)$ and also between HC(1) and both $H_2C(6)$ (an NOE not detected for **25**$\beta$) (**Fig. 23**, top left and middle).


OR
RO
O
H
H
H
α-anomer
RO
H
H
H
O
OR
β-anomer

**25** R = Ac
**27** R = Bn


HC(6)
HC(1)
HC(6)
HC(6)
HC(1)
HC(6)
4.00
4.10
4.20
4.30
4.40
ppm (t1)
4.400
4.350
4.300
4.250
4.200
4.150
4.100
4.050
ppm (t2)


OBn
BnO
O
O
OBn
BnO
O
O
1.50
1.00
0.50
0.00
2.900
2.850
2.800
2.750
2.700
ppm (t1)

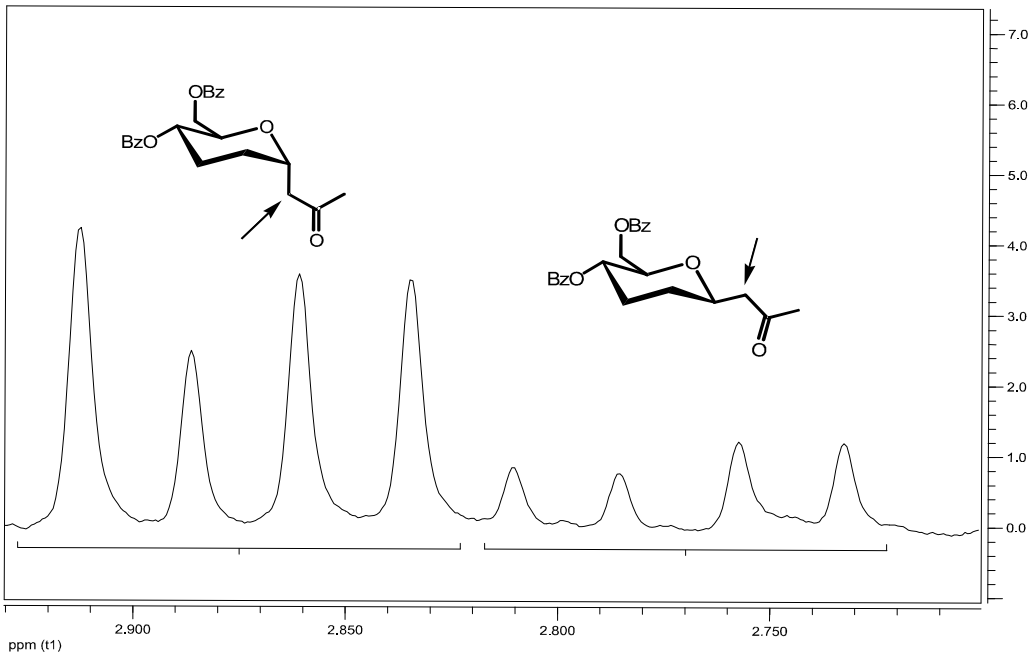



**Fig. 23** Observed NOEs for separated $\alpha$- and $\beta$-anomers of **25** and **27** (top) with characteristic NOEs between HC(1) $\rightarrow H_2C(6)$ for the $\alpha$-anomer and HC(1)$\rightarrow$HC(5) for the $\beta$-anomer, part of the NOESY of **25$\alpha$** (middle) and determination of the diastereoselectivity by integration of $^1$H-NMR data (1H of C(1)C$H_2$CO) (for **27**, **26** ).

Inspired by the *Vorbrüggen* version[59] of the *Hilbert-Johnson*-method[60] for the synthesis of nucleosides, the coupling to *C*-glycosides was also carried out from pyranosides **17**, **22**, and **23** without C(1) activation. When 1.2 eq silylenolether of acetone were used, the non C(1) activated pyranosides **17**, **22**, and **23** were treated with distilled $SnCl_4$ in dry $CH_3CN$ and the following results were obtained (**Table 4**): Bis-*O*-acetyl-pyranoside **17** produced *C*-glycoside **25** after 6 h at 25 °C in 44% yield as a diastereomeric mixture (**25$\alpha$**/**25$\beta$** = 47/53 ($^1$H-NMR)); **22** gave a diastereomeric ratio $\alpha$-D/$\beta$-D 69/31 ($^1$H-NMR) in 76% yield; and **23** gave an $\alpha$-D /$\beta$-D ratio of 18/82 ($^1$H-NMR) in 51% yield. The determination of the diastereoselectivity of **27$\alpha$**/**27$\beta$** was performed by integration of $^1$H-NMR signals of 1 H of C(1)C$H_2$CO and separation of the anomers (CC on $SiO_2$), followed by peak assignment with COSY and NOESY experiments of the separated anomers as described above for **25$\alpha$**. In the case of the bis-*O*-benzoyl-pyranoside **26**, the

separation of the anomers was unsuccessful and the structure was elucidated according to the $^3J$(HC(4), HC(5)) coupling constant (**Fig. 24**), which was for the **26**$\beta$-anomer and **25**$\beta$-anomer between 10.2 and 10.5 Hz, as expected. Further confirmation of the structure was obtained by the $\delta$ $^{13}$C ($^{13}$C-NMR) of C(1)-$CH_2$-CO, which were between 49.0 and 49.4 ppm for all $\beta$-anomers (**25**$\beta$, **26**$\beta$, **27**$\beta$) and 47.1 and 47.7 ppm for the $\alpha$-anomers (**25**$\alpha$, **26**$\alpha$, **27**$\alpha$), respectively.

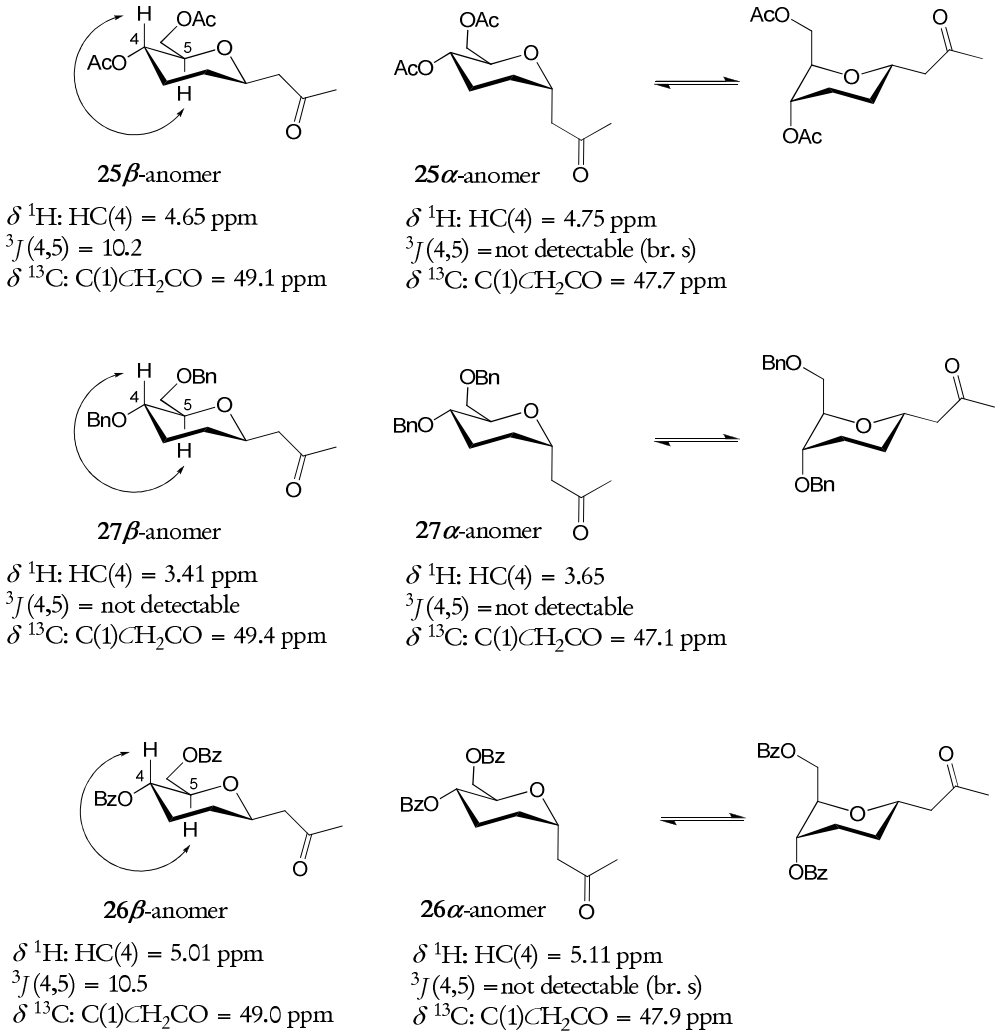



**Fig. 24** $^1$H chemical shifts of HC(4), $^3J$(HC(4), HC(5)) coupling constants (in Hertz) and $^{13}$C chemical shifts of C(1)-$CH_2$-CO for **25**, **26** and **27**. The values were obtained in the case of **25** and **27** from separated $\alpha$-D/$\beta$-D anomers, assigned by COSY and NOESY experiments, and used for the determination of anomers **26**$\alpha$, **26**$\beta$ (see text).

The addition of 3 Å molecular sieves to the reaction increased the $\alpha$-D/$\beta$-D ratio: $\alpha$-D/$\beta$-D ratios found with (without) sieves were 71/29 (47/53) for **17**, 77/23 (69/31) for **22**, and 69/31 (18/82) for **23**. These results indicate that the methanolate, formed during the oxoniumion formation, has an influence on the diastereoselectivity of the reaction. Increasing the time of the reaction from 6 h to 21 h resulted in an slightly increased diastereoselectivity ($\alpha$-D/$\beta$-D 12/88 vs. 18/82) and a decreased yield (17% vs. 63%) for **23**. These results indicate that **27**$\alpha$ rearranges under the reaction conditions to the thermodynamically favorable **27**$\beta$-anomer. Further evidence for an *in situ* $\alpha$ to $\beta$ rearrangement and the influence of a protic solvent came from the following experiment (**Fig. 25**). To a diastereomeric mixture **27**$\alpha$/**27**$\beta$ = 71/29 in dry $CH_3CN$ was added 1.4 eq distilled $SnCl_4$. After 6 h at 25 °C nearly no anomerisation occurred (**27**$\alpha$/**27**$\beta$ = 61/39). When the **27**$\alpha$/**27**$\beta$ = 71/29 mixture was subjected to the same reaction conditions in $CH_3CN$/MeOH 20:1, efficient epimerization resulted in a diastereomeric ration **27**$\alpha$/**27**$\beta$ = 34/76.

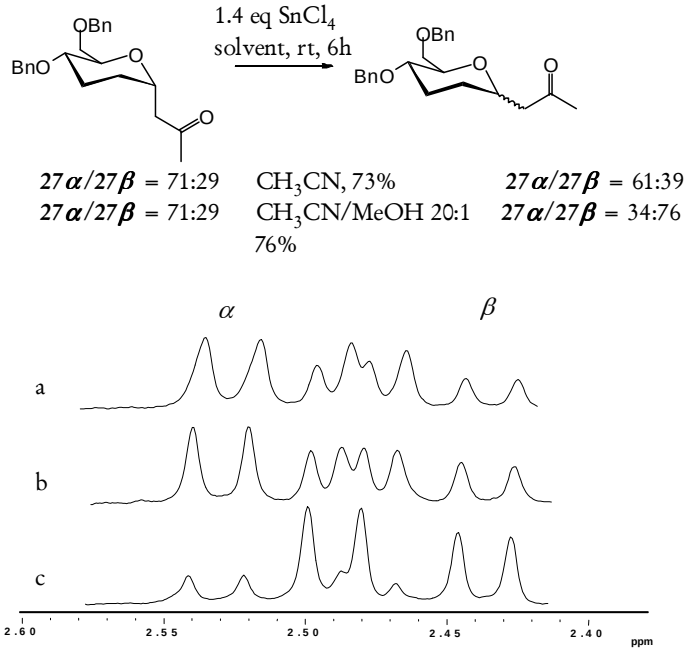
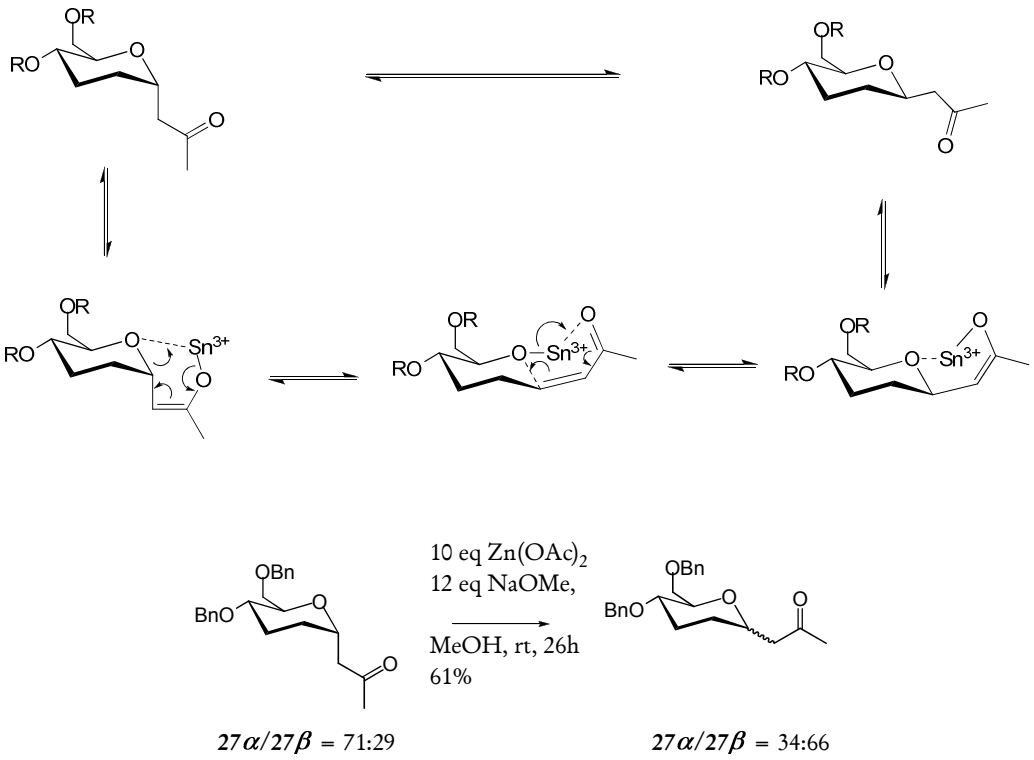



**Fig. 25** Anomerisation of **27**$\alpha$ to **27**$\beta$, followed by $^1$H-NMR of C(1)C$H_2$-CO at (a) $t$ = 0, (b) $t$ = 6 h in $CH_3CN$, (c) $t$ = 6 h in $CH_3CN$/MeOH 20:1.

The experimental evidence led to the hypothesis that the *in situ* epimerization follows this mechanism (**Scheme 6**):[61] The methanolate formed during the oxonium ion formation, induces the formation of the Sn-enolate. Due to stabilization by intramolecular chelation to the oxygen of the pyranose ring, the Z-enolate is formed, resulting in a *syn* chair-boat conformation. By a retro-*Michael* reaction, cleavage of the C(1)-O bond occurs, followed by a change in conformation. From the *anti* chair-boat conformation, the *β-C*-glycoside is formed by a *Michael* reaction. This rearrangement is less effective in the case of the ester protected sugars (**17**, **22**) compared to the ether-protected sugar **23**, probably due to a possible complexation of $Sn^{4+}$ to the ester groups (**Scheme 6**).




**Scheme 6** Proposed mechanism of the anomerisation (top) and zinc(II)-mediated epimerization according to *Zou* and coworkers (bottom).

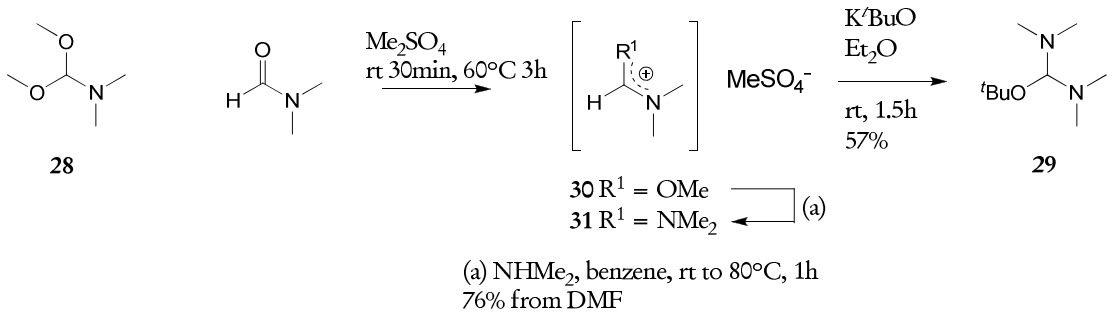
A 71/29 mixture of **27** was epimerized to 33/67 in 61% yield with a zinc(II)-mediated epimerization, reported by *Zou* and coworkers (**Scheme 6**).[62] Of all the routes to key intermediate

**27**$\beta$ the synthesis from bis-*O*-benzylpyranoside **24** was the most suitable one, due to the diastereoselectivity (**27**$\alpha$/**27**$\beta$ = 18/82), the possibility of a preparative separation of the anomers **27**$\alpha$ and **27**$\beta$ by column chromatography on $SiO_2$, and the acceptable yield (56%). Additionally the preparation of **27**$\beta$ by *C*-glycosylation did not need C(1) activation when starting from **23**. This strategy allowed us to prepare **27**$\beta$ on multi-gram scale.

### 4.3.2 Synthesis of homo-DNA-*C*-nucleosides

Derivatives of *N*,*N*-dimethylformamide (DMF) are known to react with a variety of activated methylene compounds to yield the corresponding enamino derivatives directly.[63] These enamino derivatives are activated $\beta$-ketoaldehyde equivalents (1,3-dielectrophiles), and as reported by *Schmidt* and *Hoffmann*, they undergo condensation with dinucleophiles (hydrazine, acetamidine and guanidine) to form pyrazole, 2-methyl- and 2-amino-pyrimidines, respectively.[64]

Because the commercially available *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) **28** resulted only in poor conversion of ketone **27**$\beta$ to the enaminoketone, the more reactive *tert*-butoxybis(dimethylamino)methane (*Bredereck*'s reagent) **29** was prepared from DMF in three steps according to a procedure first described by *Bredereck* and coworkers.[65] DMF was methylated with an equimolar amount of dimethylsulfate to give the *O*-methylated methylsulfate **30**,[66] which was used without purification for the synthesis of *N*,*N*,*N*',*N*'-tetramethylformamidinium-methylsulfate **31**, due to reported purification problems (for **30)** (**Scheme 7**).[66]

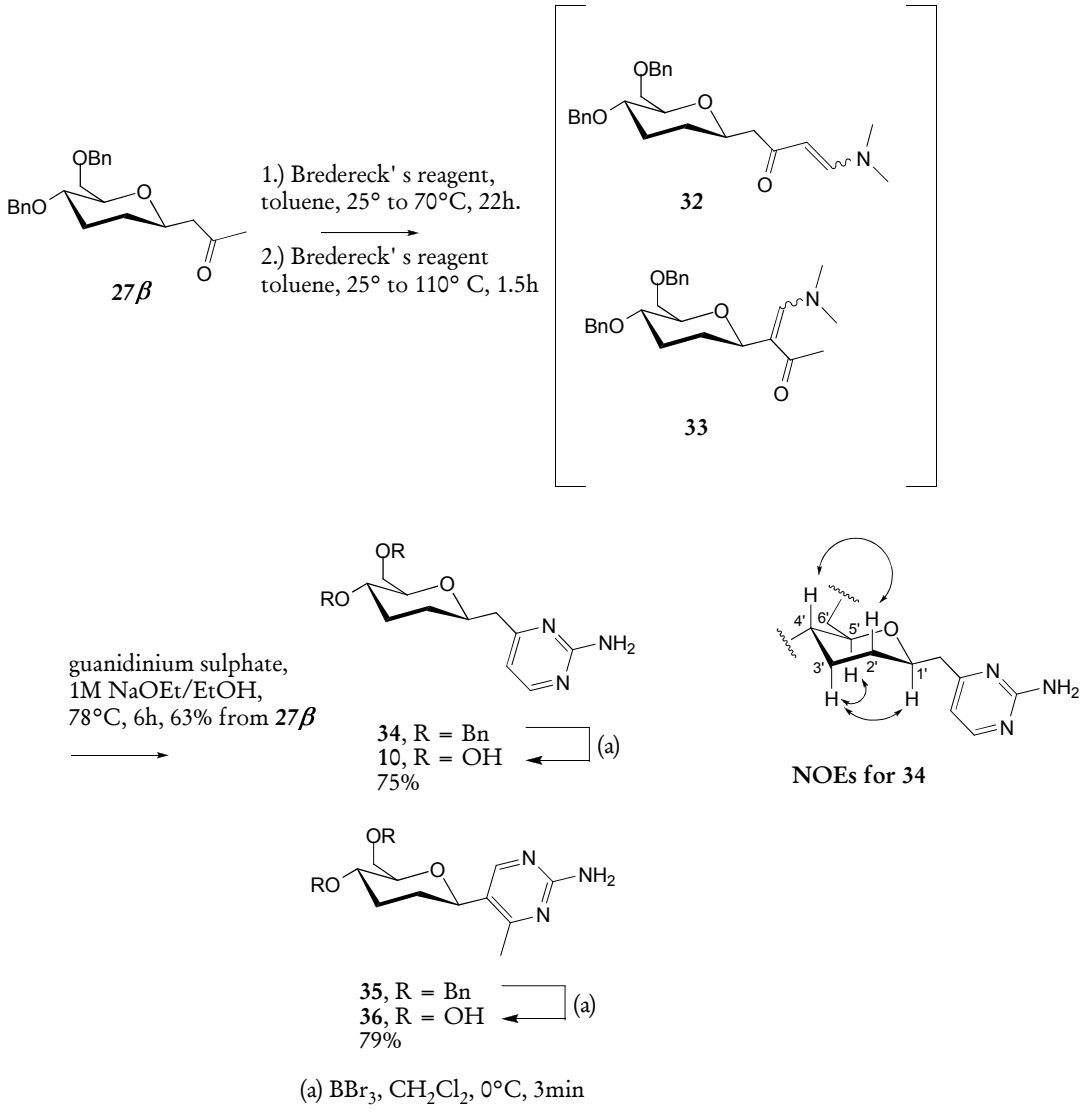



**Scheme 7** DMF-DMA **28** and synthesis of *Bredereck*'s reagent **29** from DMF.

*N*,*N*,*N*',*N*'-tetramethylformamidinium-methylsulfate **31** was obtained from **30** by treatment with a saturated solution of $Me_2NH$ in benzene as colorless low melting solid,[67] which was converted to *Bredereck*'s reagent **29** with K$^t$BuO in $Et_2O$. **29** was purified by distillation ($p$ = 11 mbar, $t$ = 60 °C).[68]

**27$\beta$** was converted to the two regioisomeric enaminoketones **32** and **33** with *Bredereck*'s reagent **29** in toluene. Condensation with guanidinium sulfate (or guanidinium chloride) provided a mixture of homo-DNA-$\beta$-*C*-nucleosides **34** and **35** in a 3:1 ratio (for both guanidinium sulphate and guanidinium chloride) with a yield of 63% (58% with guanidinium chloride) (from **27$\beta$**). Homo-DNA-*C*-alkyl-nucleoside **34** was separated from homo-DNA-*C*-aryl-nucleoside **35** by column chromatography on neutral $Al_2O_3$ (*Brockmann* activity III). Attempts to separate **34** and **35** on $SiO_2$ failed. From mixed fractions of **34** and **35**, enriched in **35** (**34**/**35** = 1:2), pure **35** was obtained by recrystallisation from EtOAc/hexane 2:1, due to the good crystallization properties of homo-DNA-*C*-aryl-nucleoside **35**. No occurrence of anomerisation under the basic reaction conditions of the condensation was deduced by COSY and NOESY experiments of *C*-nucleoside **34** (NOEs: HC(1')/HC(6')→$H_{ax.}$C(3'), $H_{ax.}$C(2')→HC(4')/HC(5'), and HC(5')/HC(4')→$H_{ax.}$C(3'). In contrast, a process of deprotonation of the acidic carbonyl-$\alpha$-protons followed by a ring-opening of the pyranose ring was reported by *Hoffmann* and *Schmidt* for the $\alpha$-anomer of the corresponding *O*-benzylated glucopyranosyl-ketone (**Fig. 26**).[64]

Hydrogenolytic deprotection of homo-DNA-*C*-nucleosides **34** and **35** failed under various conditions (Pd(C) or Pd(C) with up to 60% $Pd_{black}$ (precipitated Pd) in MeOH/EtOAc 3:1). Protection of the primary amine in **34** as the isobutyric amide[69] or as the benzyl-amide[32a] did not facilitate the deprotection of the bis-*O*-benzylether. Ultimately, homo-DNA-*C*-nucleosides **34** and **35** were deprotected to the 4',6'-diols **10** and **36**, following a procedure by *Hart* et al.,[70] under *Lewis* acidic conditions with $BBr_3$ in $CH_2Cl_2$ with a yield of 75% (**10**) and 79% (**36**), respectively.



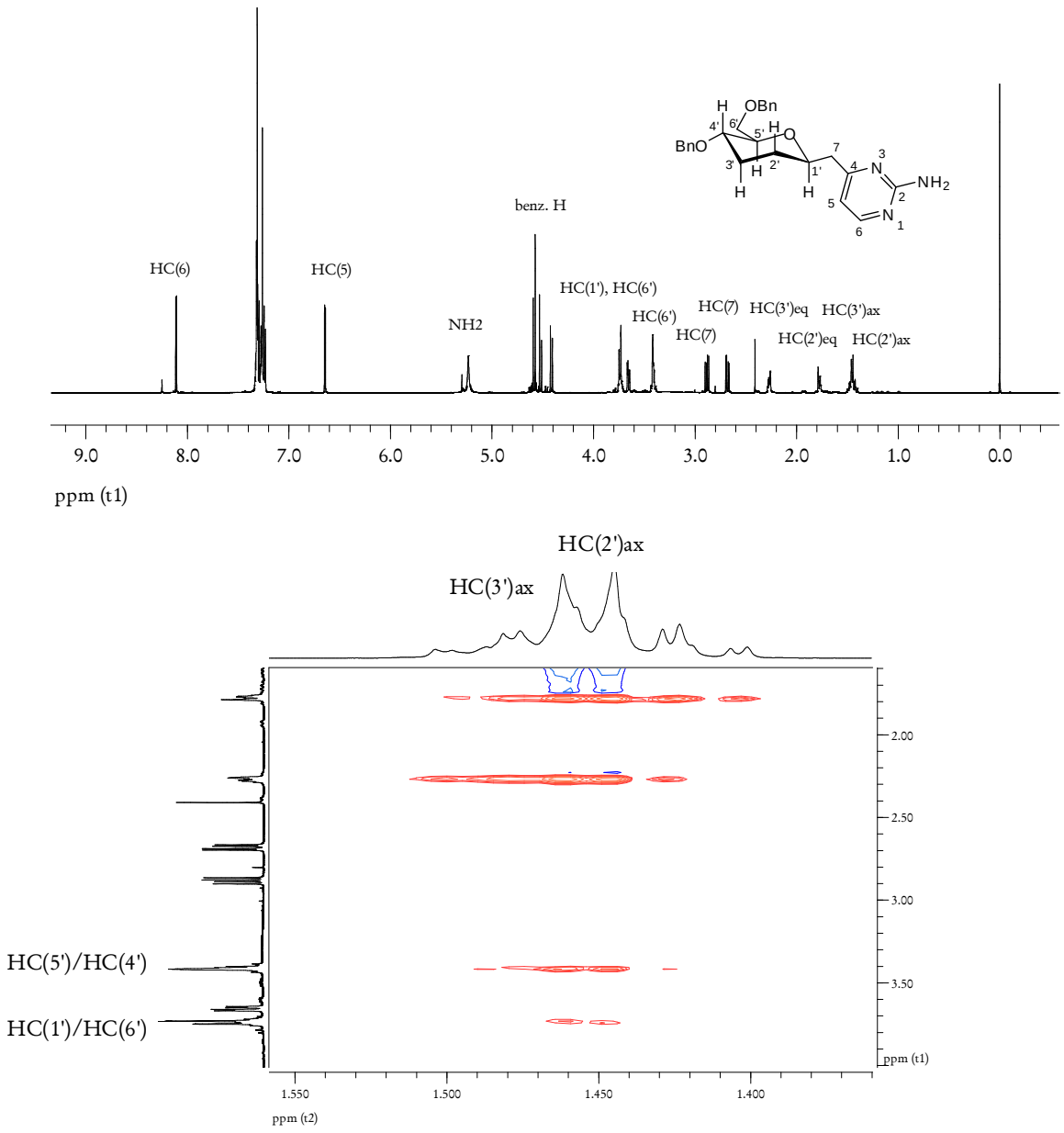
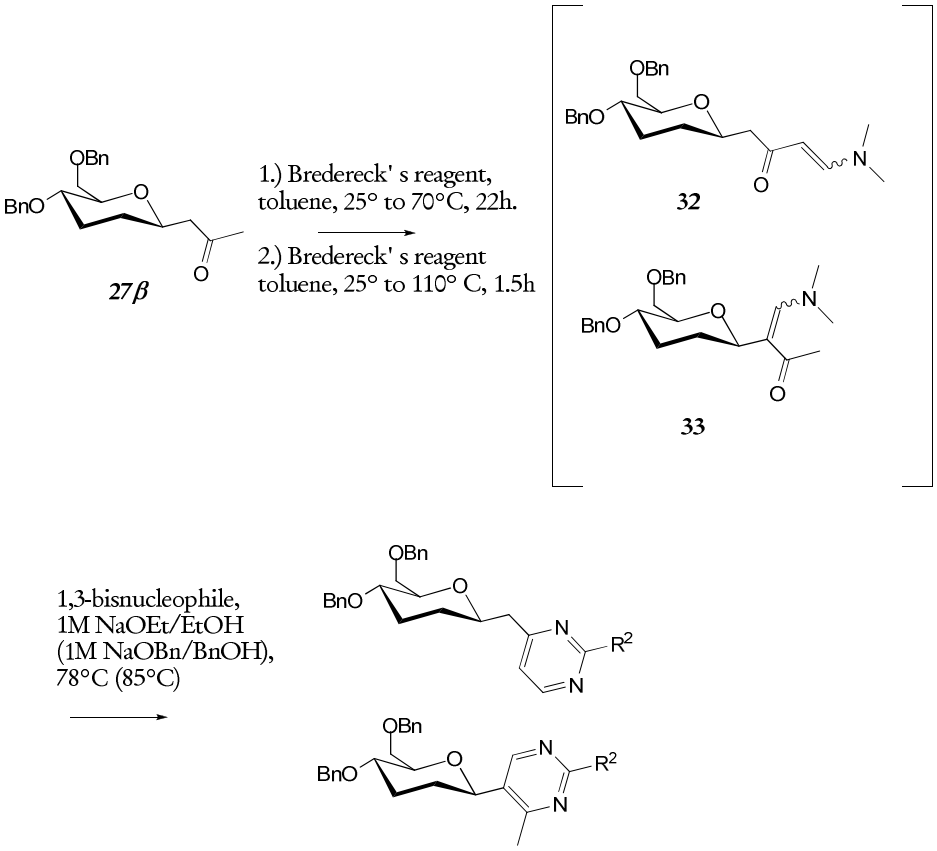



**Fig. 26** Synthesis of fully unprotected homo-DNA-*β-C*-nucleosides **10** and **36** from *β-C*-glyco-pyranoside **27*β*** and observed NOEs in the NOESY experiment for **34** excluding a known anomerisation reported by *Hoffmann* and *Schmidt* under basic condensation conditions (top).[64] $^{1}$H-, and NOESY spectrum of **34** (bottom).

Condensation with poorer 1,3-bisnucleophiles than guanidinium sulphate (*O*-methylisourea hemisulfate or *S*-methylisothiourea hemisulfate) reduced the yields of the

condensation under the same reaction conditions and produced higher ratios of the homo-DNA-*C*-aryl-nucleoside vs. homo-DNA-*C*-alkyl-nucleoside as mixtures with -OEt substitutents at C(2) of the pyrimidine, resulting from an $S_NAr$ reaction with the base as nucleophile (**Table 5**). This observation was applied in a second strategy to prepare 2-oxo-homo-DNA-*C*-alkyl-nucleoside **11** from the corresponding 2-*O*-benzyl-pyrimidine derivative, which should be prepared by a one-pot reaction (condensation, $S_NAr$ reaction) in NaOBn/BnOH. Due to poor condensation yields (10 to 26%) for both *S*-methylisothiourea hemisulfate and *O*-methylisourea hemisulfate and the preferred formation of the undesired homo-DNA-*C*-aryl-nucleoside, this alternative strategy for the synthesis of homo-DNA-*C*-alkyl-nucleoside **11** was discarded (**Table 5**). No condensation was observed with urea.



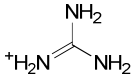
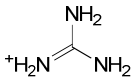
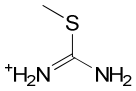
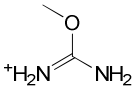
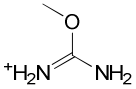
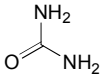
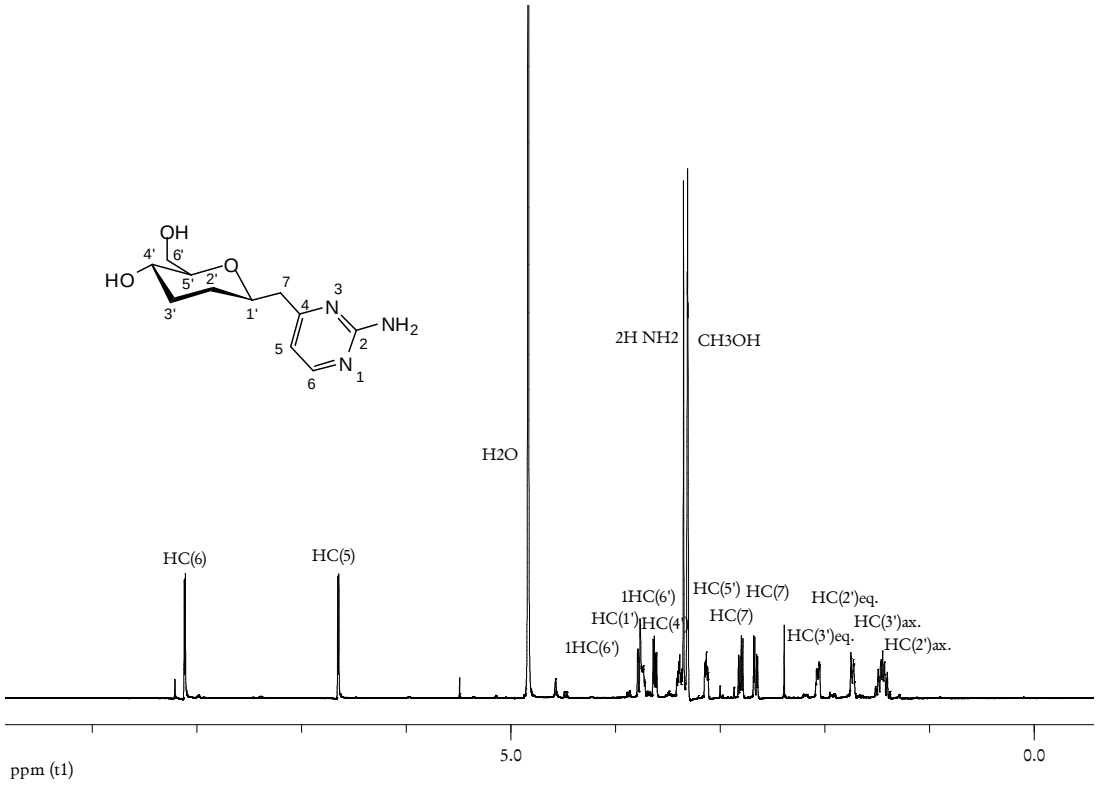
| $X^-$ | Nucleophile | Solvent/ base | Aryl-*C*-nucleoside/alkyl-*C*-nucleoside | Yield (from 27$\beta$) | $R^2$ |
|---|---|---|---|---|---|
| 0.5 $SO_4^{2-}$ |  | EtOH/ NaOEt | 1:3 (isolated) | 63% | $NH_2$ |
| $Cl^-$ |  | EtOH/ NaOEt | 1:3 (isolated) | 58% | $NH_2$ |
| 0.5 $SO_4^{2-}$ |  | EtOH/ NaOEt | 3:2 ($^1$H-NMR) | 26% | OEt |
| 0.5 $SO_4^{2-}$ |  | EtOH/ NaOEt | 3:1 ($^1$H-NMR) | 15% | OEt |
| 0.5 $SO_4^{2-}$ |  | BnOH/ NaOBn | 4:1 ($^1$H-NMR) | 10% | OBn |
| - |  | EtOH/ NaOEt | - | - | - |

**Table 5** Condensation of enaminoketones **32** and **33** with *O*-methyl isourea hemisulfate, *S*-methylisothiourea hemisulfate and urea, respectively.



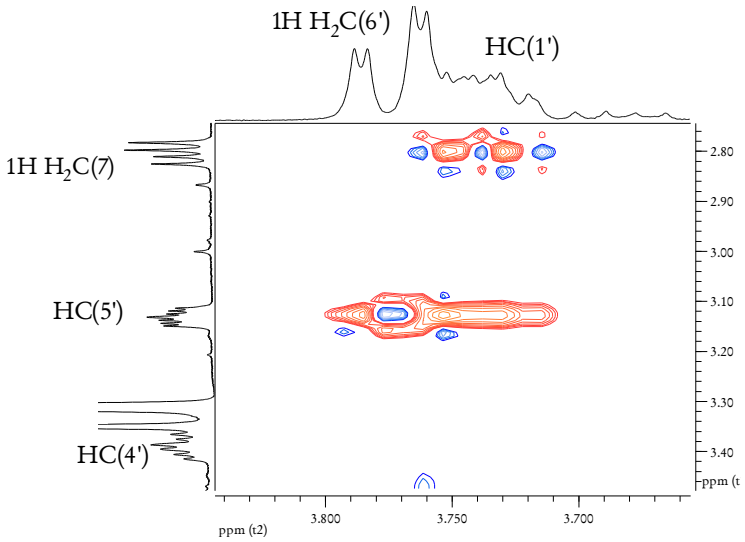



**Fig. 27** [1]H-NMR (peaks assigned according to COSY experiment) (top), and observed NOE between HC(1')-HC(5') (bottom) important for the assignment of *β*-configuration for homo-DNA-*β*-*C*-alkyl-nucleoside **10** (all spectra were measured in $d^4$-MeOD at 300 K and 500 Mhz).

To exclude the possibility that an anomerisation of homo-DNA-*β*-*C*-alkyl-nucleoside **10** had occurred under the strongly *Lewis* acidic conditions necessary for the deprotection of the bisbenzylether in **34** COESY and NOESY experiments of **10** were performed. They supported the *β*-D-configuration by displaying the characteristic HC(1')→HC(5') NOE (**Fig. 27**).

The same strategy was applied for the determination of the fully deprotected homo-DNA-*β*-*C*-aryl-nucleoside **36**. The characteristic HC(1')→HC(5') NOE of the *β*-D-configuration was observed here as well (**Fig. 28**).

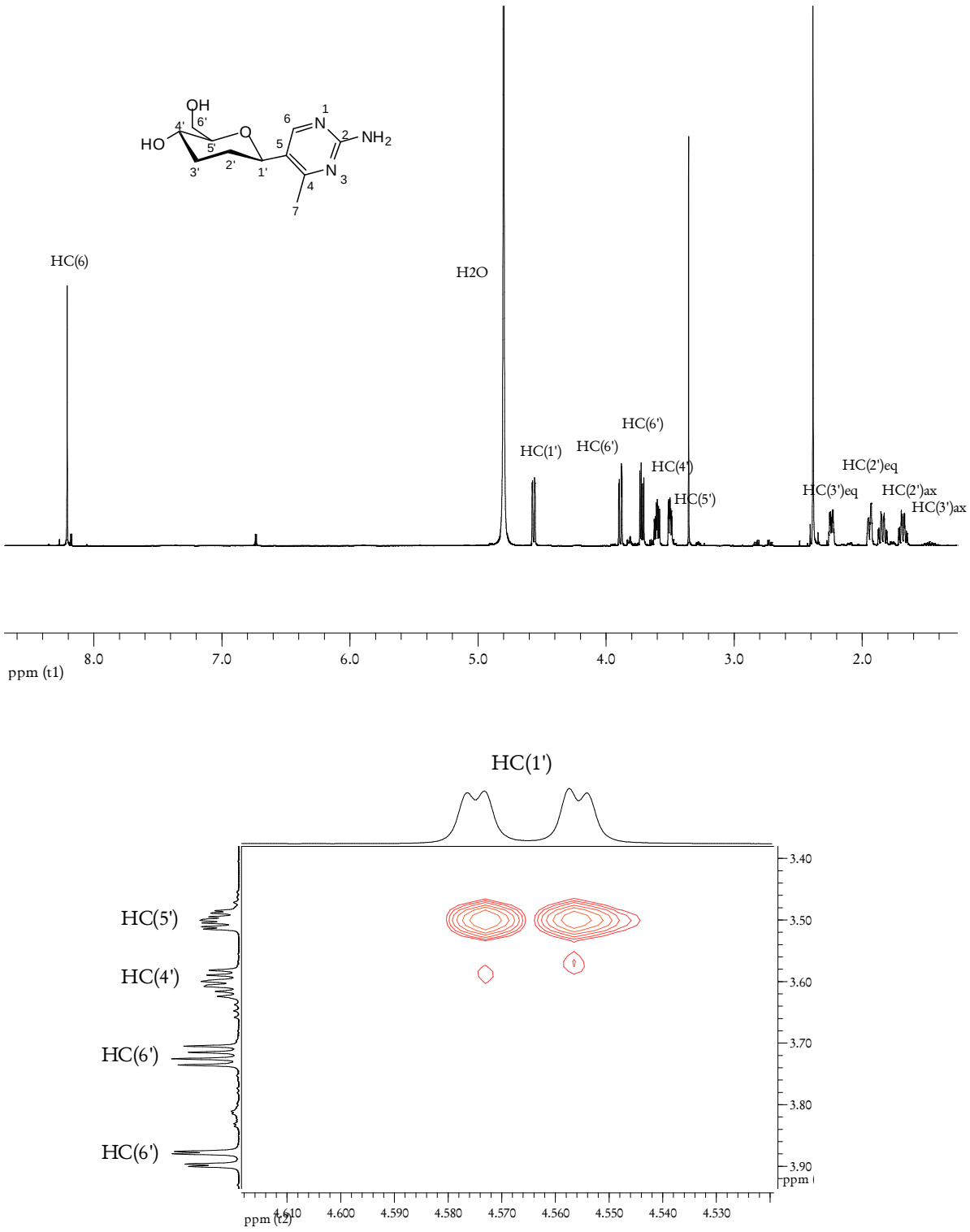
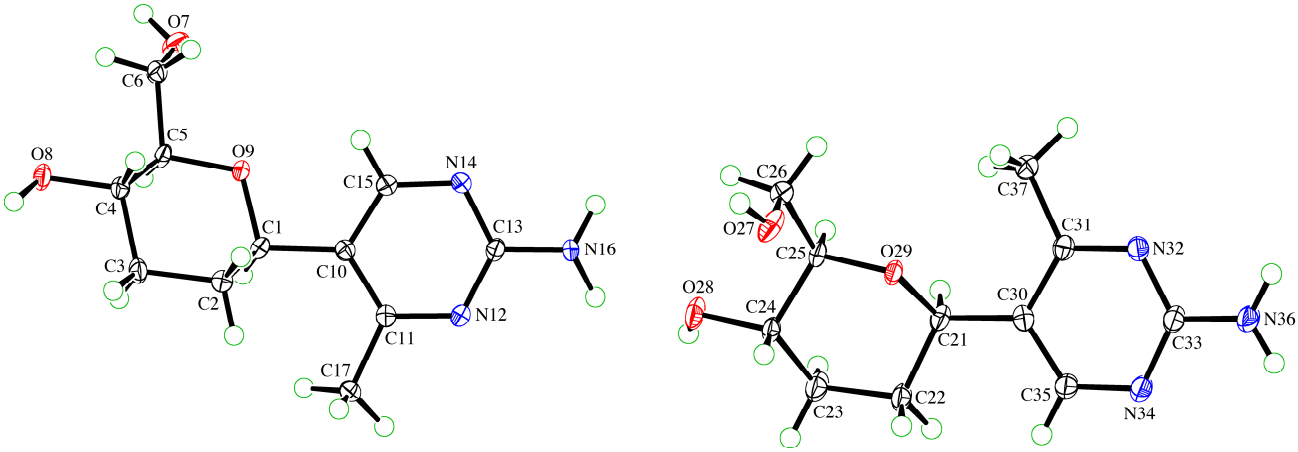



**Fig. 28** $^{1}$H-NMR (peaks assigned according to COSY experiment) (top), and observed NOE between HC(1')-HC(5') (bottom) important for the assignment of *β*-configuration for homo-DNA-*β*-*C*-aryl-nucleoside **36** (all spectra measured in $D_2O$ at 300 K and 600 Mhz).

Homo-DNA-*β*-*C*-aryl-nucleoside **36** was crystallized from $D_2O$, resulting in colorless needles suitable for X-ray crystal structure analysis. In the crystal of **36** two conformations, different in the torsion angles $\chi$(O(9/29)-C(1/21)-C(10/30)-C(11/31)) and $\gamma$(O(7/27)-C(6/26)-C(5/25)-C(4/24)), occur in a 1:1 ratio. In conformer **36A** (**Fig. 29**) the values for $\chi$ and $\gamma$ are -165.5°, -161.6° (antiperiplanar); in the other conformer B, -54.0° and 58.1 (synclinal), respectively. Intermolecular contacts between the bases in **36** occur with conformational recognition: the nucleobase of conformer **36A** forms exclusively two hydrogen bonds to two nucleobases of nucleosides with the same conformation. Conformer **36B** only forms four hydrogen-bonds to two nucleobases of nucleosides with the same **36B** conformation. Further intermolecular contacts occur between HO(7) of conformer A and HO(28) of conformer B, and between HO(8) of conformer A and HO(27) of conformer B, forming twelve-membered loops and extended …**36A**…**36B**…**36A**…**36B**… chains. The x-ray structure of of **36** proved the *β*-configuration of **36**, which had also been determined by NOESY experiments (**Fig. 29**).



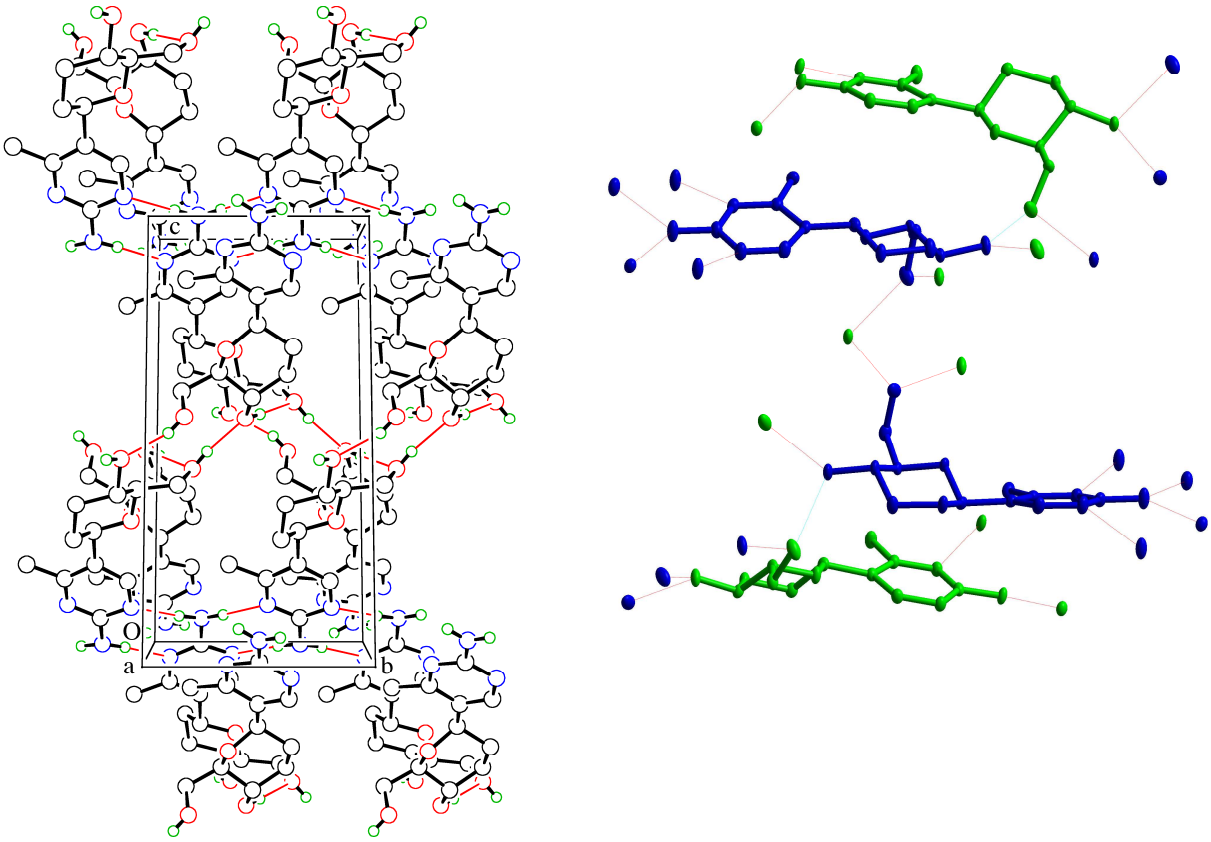
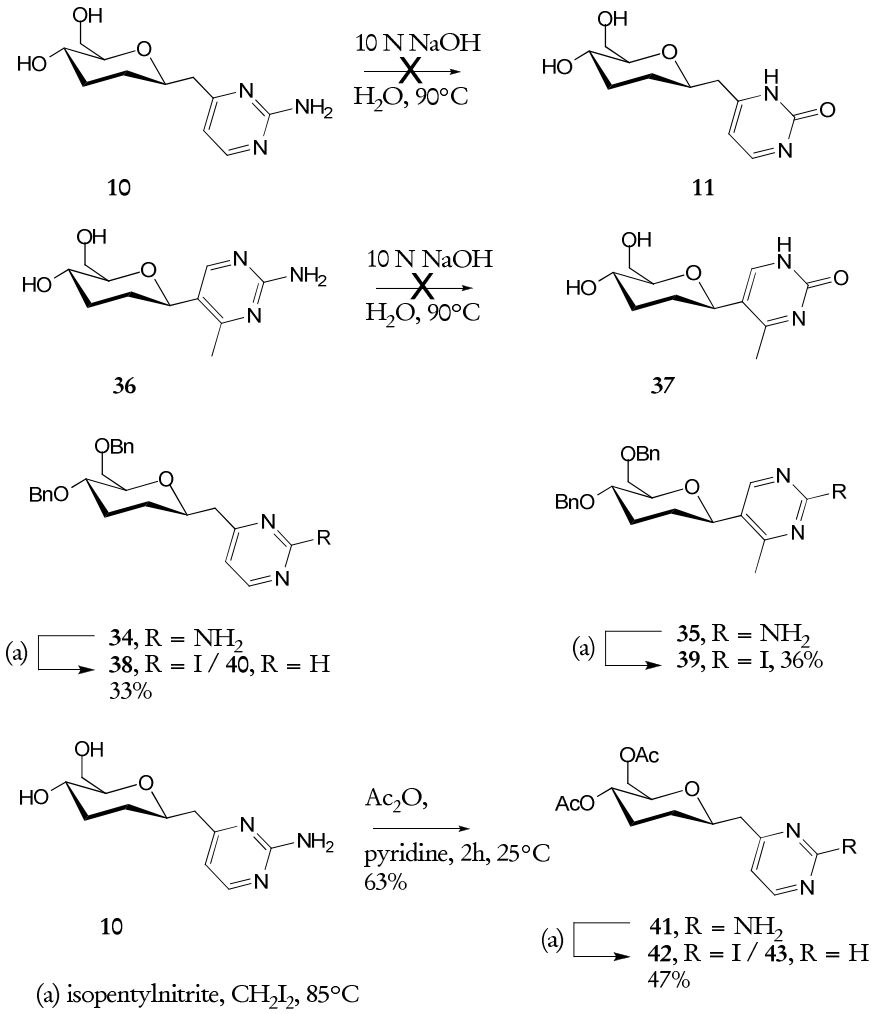


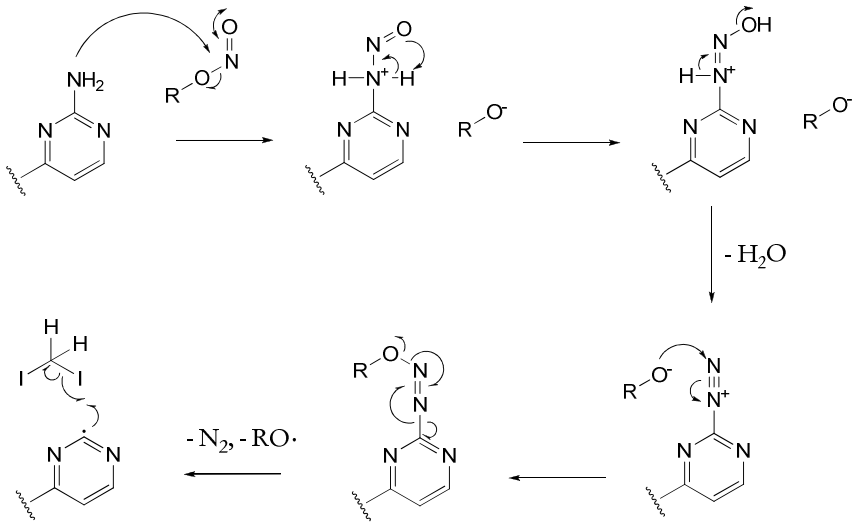

**Fig. 29** X-ray structures of conformer **36A** (top left) and **36B** (top right) of homo-DNA-*β*-*C*-aryl-nucleoside **36** and packing of **36** (bottom) with conformer **36A** in green and **36B** in blue, respectively.

Under basic hydrolysis conditions (10 N NaOH in $H_2O$), reasonable amounts of the corresponding pyrimidine-2-ones **11** and **37** were not obtained from homo-DNA-*β*-*C*- nucleosides **10** nor **36**, although the product was detected in solution by ESI-MS (**Scheme 8**). Acidic diazotation with $NaNO_2$, according a procedure described by *Schulz* and *Pfleiderer*,[71] failed for both the benzyl-protected homo-DNA-*β*-*C*-alkyl-nucleoside **34** and the homo-DNA-*β*-*C*-aryl-nucleoside **35**, so did many other acidic diazotations. The first method successfully applied in the conversion of **34** to the corresponding 2-iodopyrimidine-nucleoside **38** was a procedure of *Loren*,[72] which used an isopentylnitrite in benzene with $I_2$, as an iodine-radical donor, trapping the intermediately formed pyrimidine-2-radical (**Scheme 9**). The yield of the reaction was increased

from 21% to 33% by replacing $I_2$ with diiodomethane as iodine-radical donor, according to a procedure described by *Nair* and *Richardson*.[73] This preparation of 2-iodo-pyrimidine-nucleosides was also successfully applied in the case of the homo-DNA-*β*-*C*-aryl-nucleoside **35** with a yield of 36% for the 2-iodopyrimidine-*β*-*C*-aryl-nucleoside **39**. In the synthesis of 2-iodopyrimidine-*β*-*C*-alkyl-nucleoside **38**, the deaminated product **40** was isolated as a side product. To exclude the possibility of radical hydrogen abstraction from the benzylic position of the benzyl protecting groups, we prepared from the fully deprotected homo-DNA-*β*-*C*-alkyl-nucleoside **10**, according to a procedure of *Martini* and *Bredereck,*[74] the bis-*O*-acetylated derivative **41** in 63% yield, which was converted to the 2-iodopyrimidine-nucleoside **42** according the procedures described for **38** and **39**. Although the yield could be increased in the synthesis of **42** (47%), compared to the bis-*O*-benzyl-analogue **38** (33%), the deaminated nucleoside **43** was also observed in the preparation of **41**.

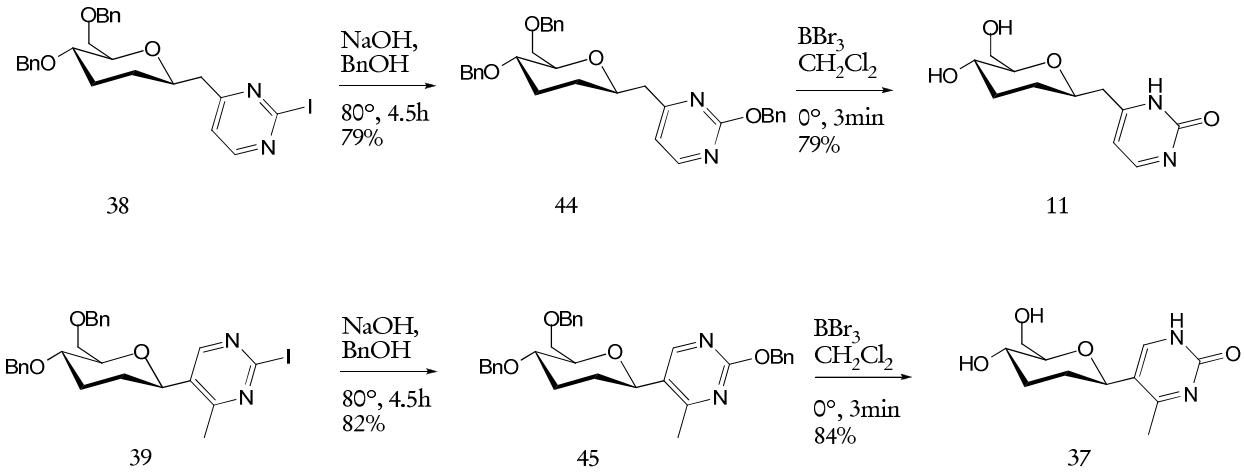



**Scheme 8** Synthesis of 2-iodo-homo-DNA-*β*-*C*-alkyl-nucleoside **38** and 2-iodo-homo-DNA-*β*-*C*-aryl-nucleoside **39**.




**Scheme 9** Mechanism of the formation of the 2-iodo-pyrimidine nucleosides according to *Nair* and *Richardson*.[75]

Solvolysis of 2-iodopyrimidin-*C*-alkyl-nucleoside **38** and 2-iodopyrimidin-*C*-aryl-nucleoside **39** in with NaOH in BnOH following a procedure by *Camaioni* et al.[76] resulted in good yields (79% for **38** and 82% for **39**) of the corresponding 2-*O*-benzylpyrimidin-*C*-alkyl- and -*C*-aryl-nucleosides **44** and **45**. Applying the conditions reported above for the deprotection of **34** and **35** resulted in fully deprotected 2-oxopyrimidine-homo-DNA-$\beta$-*C*-alkyl-nucleoside **11** and -*C*-aryl-nucleoside **37** with yields of 79% and 84%, respectively (**Scheme 10**).




**Scheme 10** Preparation of fully unprotected 2-oxopyrimidin-homo-DNA-$\beta$-*C*-alkyl-nucleoside **11** and -*C*-aryl-nucleoside **37**.

To exclude the occurrence of an anomerisation of 2-oxopyrimidine-homo-DNA-$\beta$-*C*-alkyl-nucleoside **11** and *C*-aryl-nucleoside **37** under the strongly *Lewis* acidic conditions necessary for the deprotection of the trisbenzylether in **44** and **45,** respectively, COSY and NOESY experiments were performed for **11** and **37**. The characteristic HC(1')→HC(5') NOES for the $\beta$-D-configuration was observed for both nucleosides (**Fig. 30**, **Fig. 31**).

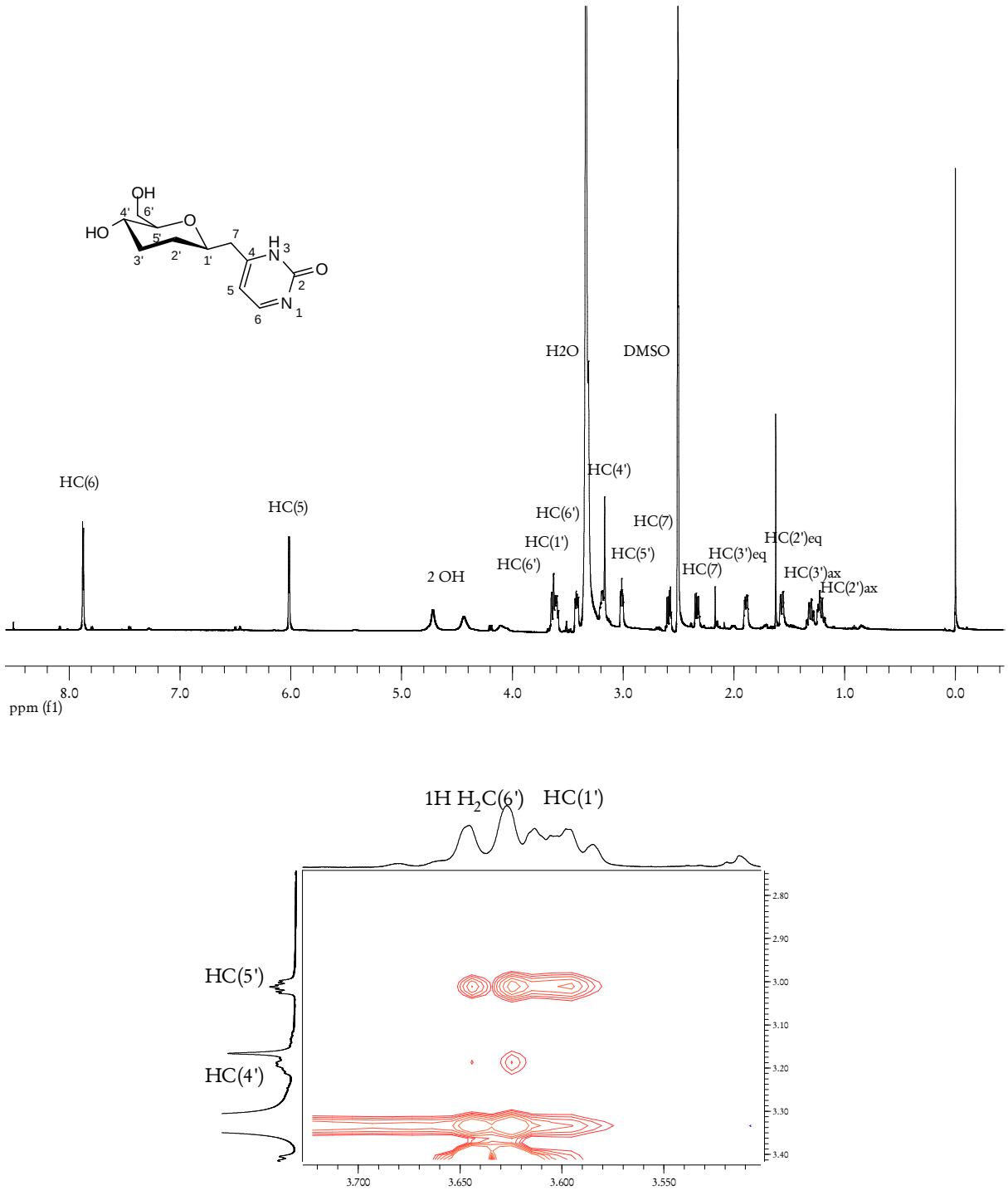



**Fig. 30** $^{1}$H-NMR (peaks assigned according to COSY experiment) (top), and observed NOE between HC(1')-HC(5') (bottom) important for the assignment of *β*-configuration for 2-oxopyrimidine-homo-DNA-*β*-*C*-alkyl-nucleoside **11** (all spectra were measured in d$^{6}$-DMSO at 300 K and 600 Mhz).

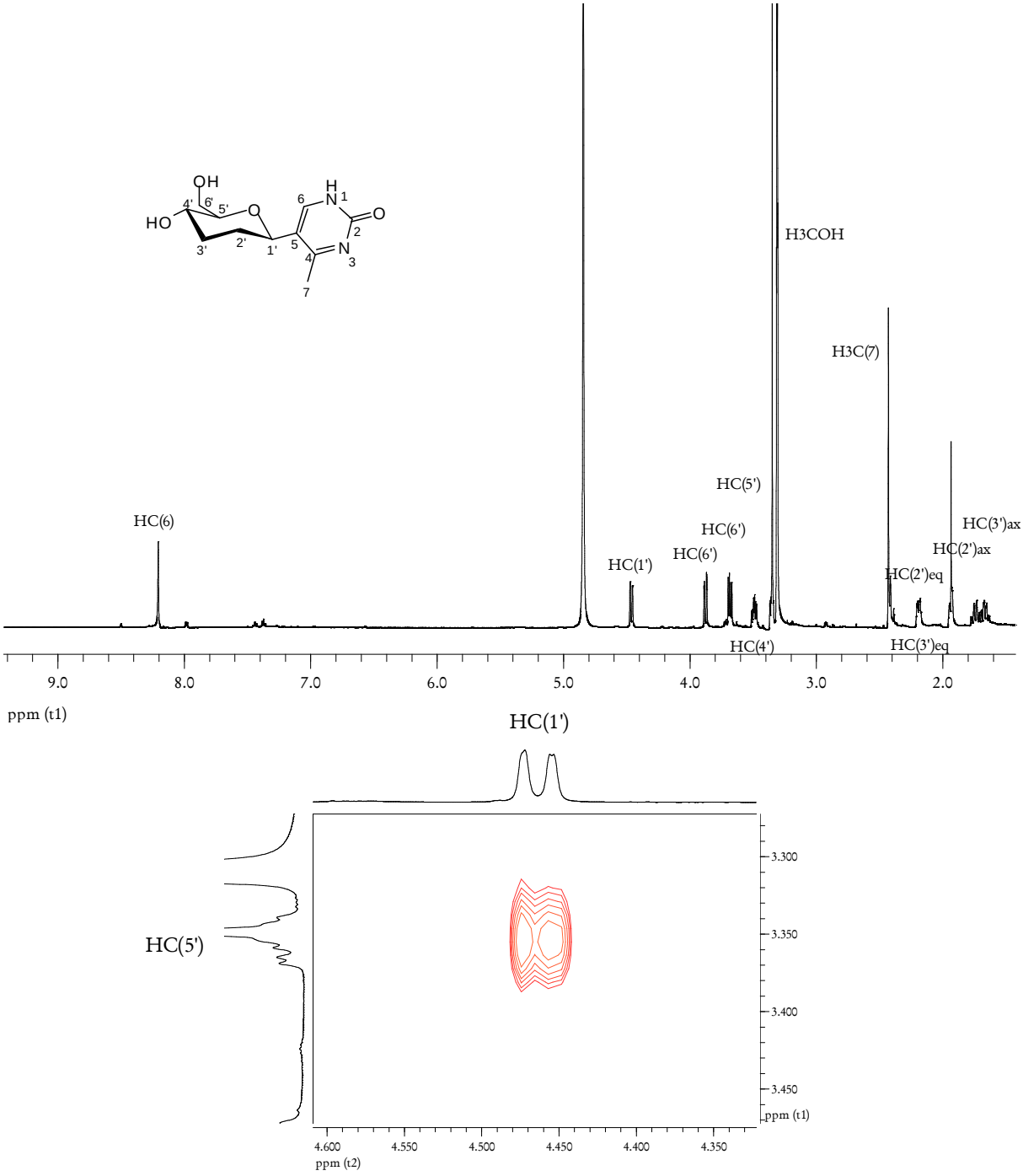
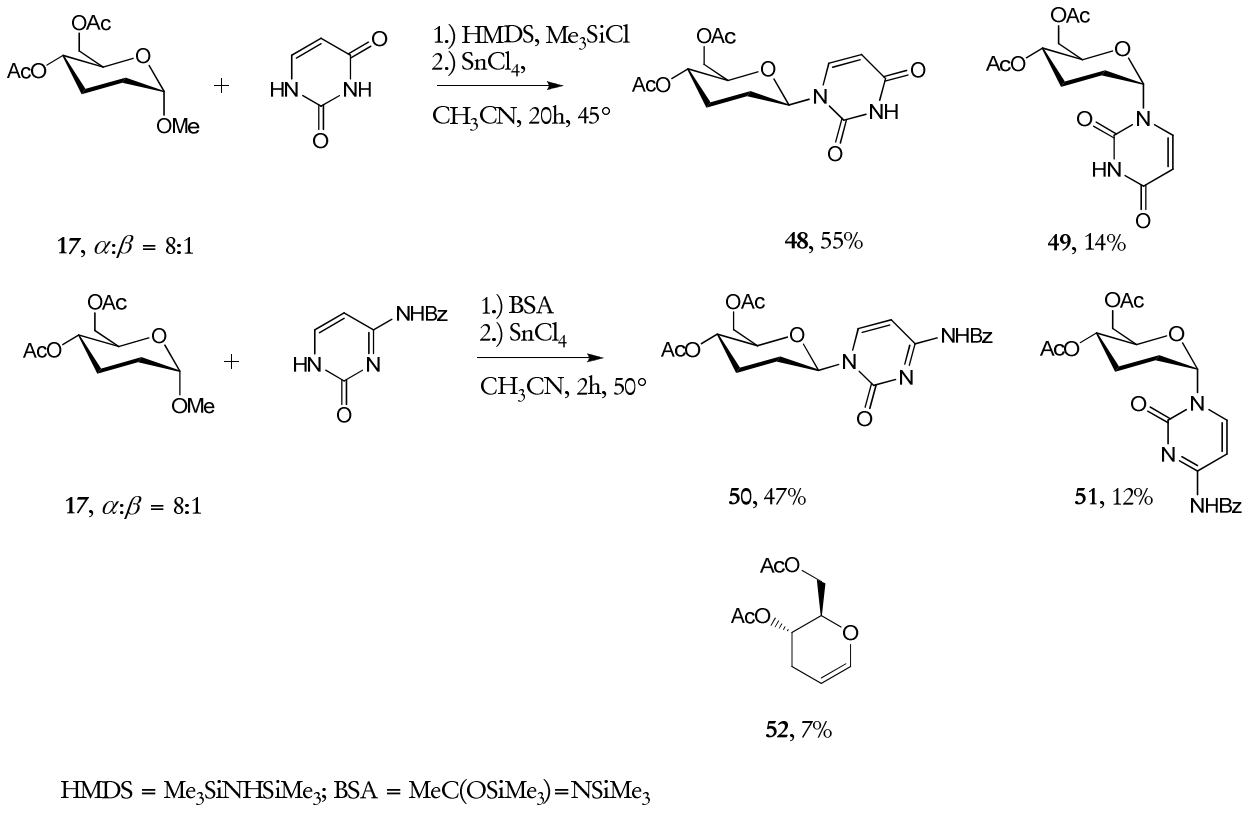



**Fig. 31** $^{1}$H-NMR (peaks assigned according to COSY experiment) (top), and observed NOE between HC(1')-HC(5') (bottom) important for the assignment of *β*-configuration for 2-oxopyrimidine-homo-DNA-*β*-D-*C*-aryl-nucleoside **37** (all spectra were measured in d$^{4}$-MeOD at 300 K and 600 Mhz).

### 4.3.3 Synthesis of homo-DNA-nucleosides with natural nucleobases

The synthesis of the homo-DNA-nucleosides with natural nucleobases followed by some modifications the methodologies described by *Eschenmoser* and coworkers,[32a] with the key step being a *Hilbert-Johnson*-method[60] for nucleoside-synthesis, modified by *Vorbrüggen* and coworkers.[59a] This variant of the nucleoside synthesis forms the nucleosidic bond between the sugar and the per-silylated heterocyclic base under *Lewis* acid catalysis and does not require any activated leaving group at the anomeric centre. Because it is reported that the configuration at the anomeric centre of **17** influenced neither the product distribution nor the yields of the nucleoside formation, **17** was used as an anomeric mixture.

The persilylated heterocyclic bases were prepared *in situ* in the reaction mixture. In a typical experiment for the preparation of a pyrimidine-nucleoside **48**, uracil was suspended in a solution of methyl pyranoside **17** in dry $CH_3CN$. After adding an excess of silylating reagents (hexamethyldisilazane (HMDS) and chloro(trimethyl)silane ($Me_3SiCl$)) followed by $SnCl_4$, which resulted in a homogenous reaction mixture, the reaction was heated to 45°C for 20 h. This procedure resulted in $\beta$-D-nucleoside **48** as the major product (55%) with 14% of the corresponding $\alpha$-D-anomer **49** (**Scheme 11**) after aqueous work-up and separation of the anomers by column chromatography. The corresponding cytosine derivatives were prepared in an analogous approach by silylating commercially available $N^4$-benzoylcytosine *in situ* with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) in a solution of pyranoside **17** in $CH_3CN$ followed by the addition of $SnCl_4$ and heating to 50° C for 2 h, resulting in a chromatographically separable $\beta$-D/$\alpha$-D-mixture in a total yield of 57%, with the thermodynamically more stable $\beta$-D-anomer being the major product. As a side product from the reaction mixture 7% of enolether **52** was isolated.



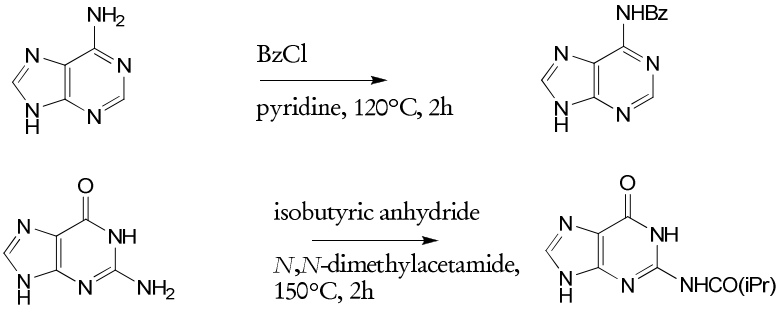

**17**, $\alpha$:$\beta$ = 8:1 — **48**, 55% — **49**, 14%

**17**, $\alpha$:$\beta$ = 8:1 — **50**, 47% — **51**, 12%

**52**, 7%

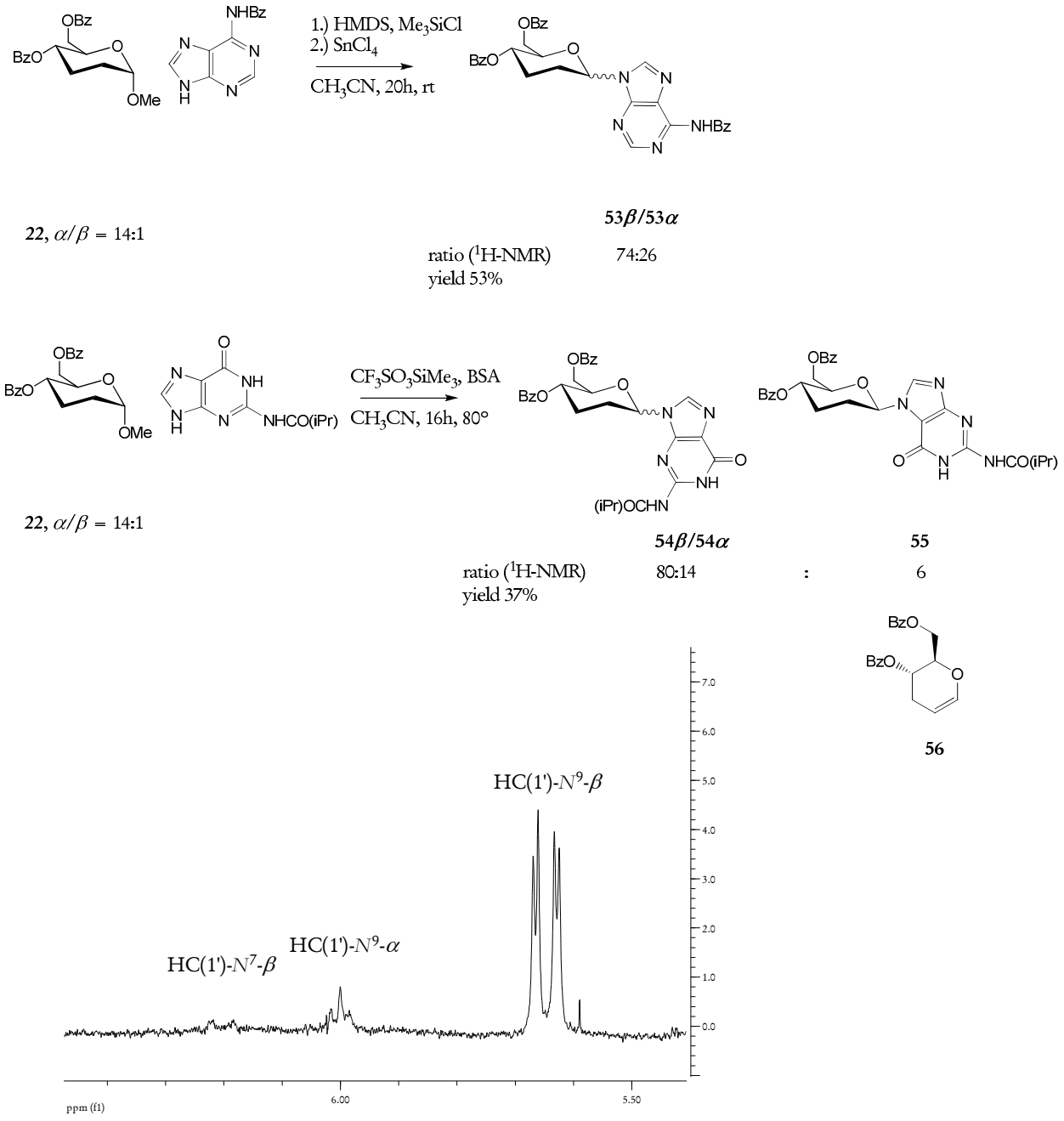
**Scheme 11** *Vorbrüggen* nucleoside synthesis for the preparation of the $\beta$-D-anomers of homo-DNA-uracil and homo-DNA-cytosine-nucleosides **48** and **50**, respectively.

$N^6$-benzoyladenine and $N^2$-isobutyrylguanine were prepared from adenine and guanine, respectively, according procedures described by *Bullock* et al.[77] for adenine and *Benner* and coworkers for guanine.[78] BzCl (or isobutyric anhydride) was added to a suspension of adenine in pyridine or guanine in *N*,*N*-dimethylacetamide at rt. The reaction was heated to 120 °C (150 °C) for 2 h. Aqueous workup, followed by recrystallisation from boiling EtOH/$H_2O$ 1:1, resulted in 72% $N^6$-benzoyladenine and 85% $N^2$-isobutyrylguanine (**Scheme 12**).




**Scheme 12** Preparation of $N^6$-benzoyladenine and $N^2$-isobutyrylguanine according to *Bullock* et al.[77] and *Benner* and coworkers.[78]

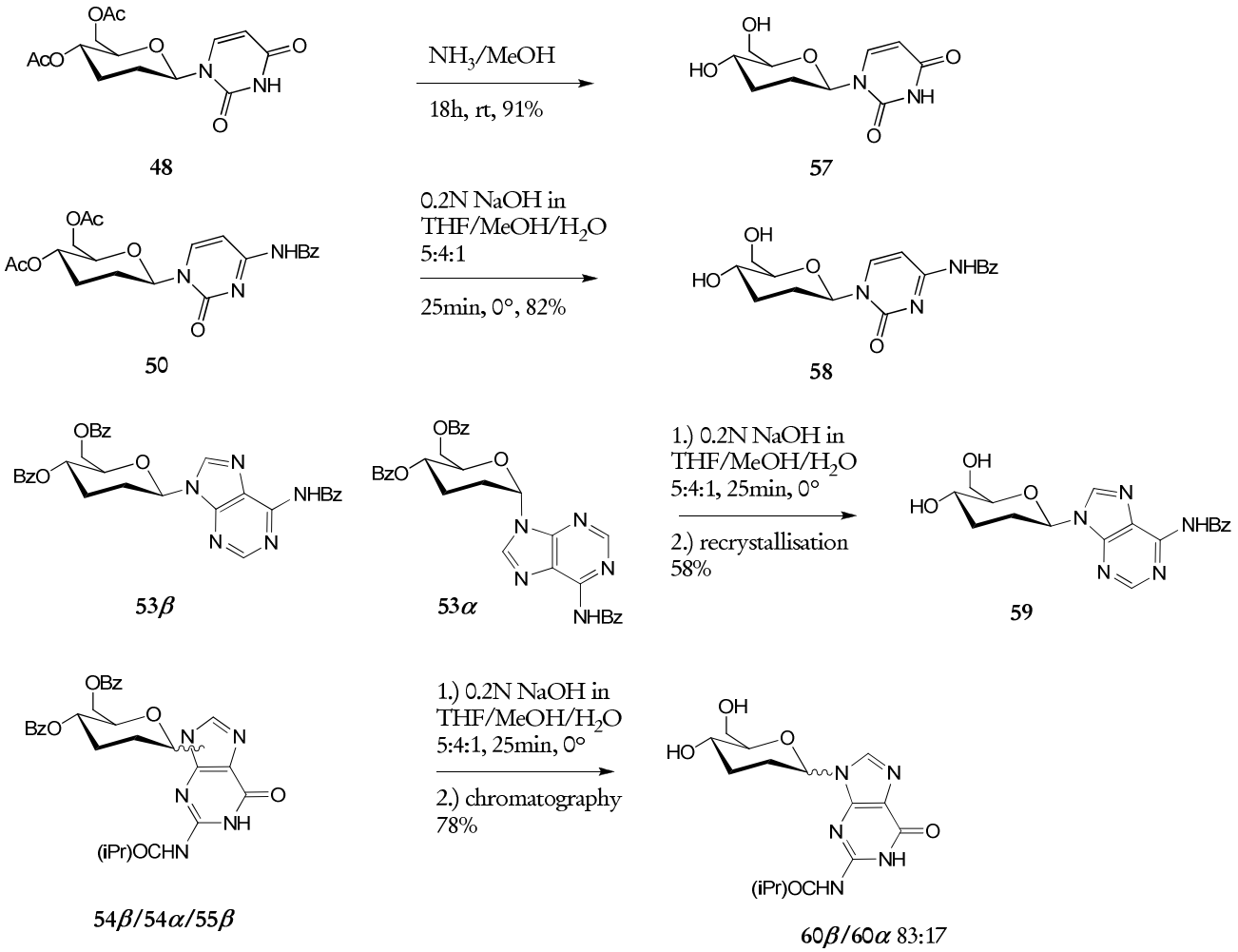
The preparation of the $\beta$-D-homo-DNA-purine nucleosides **53$\beta$** and **54$\beta$** was more challenging than the preparation of the $\beta$-D-homo-DNA-pyrimidine nucleosides **48** and **50** (**Scheme 13**): homo-DNA-adenine-nucleoside **53** was prepared from a solution of **22** and $N^6$-benzoyladenine in $CH_3CN$ by the addition of hexamethyldisilazane/$Me_3SiCl$, followed by $SnCl_4$, which resulted in a homogenous solution that was worked up after a reaction time of 20 h at rt (**Scheme 13**). After column chromatography, a **53$\beta$**/**53$\alpha$** 74:26 mixture was obtained in a yield of 53%. Further attempts to separate the anomers **53$\beta$** and **53$\alpha$** by chromatography and crystallization failed. Homo-deoxyguanosine **54** was prepared from the *in situ* per-silylated $N^2$-isobutyrylguanine with dibenzoylpyranoside **22** under $CF_3SO_3SiMe_3$ catalysis in refluxing $CH_3CN$. The reaction resulted in a mixture of $N^9$-$\beta$-D/$N^9$-$\alpha$-D/$N^7$-$\beta$-D in an 80:14:6 ratio with a total yield of 37%. Both couplings to the $\beta$-D-homo-DNA-purin-nucleosides resulted in formation of enolether **56** as side product, most likely resulting from an intramolecular stabilization of the intermediate oxonium ion.




**Scheme 13** *Vorbrüggen* nucleoside synthesis for the preparation of the of homo-DNA-adenine and homo-DNA-guanine-nucleosides **53** and **54**, respectively (top) and determination of the $N^9$-$\beta$-D/$N^9$-$\alpha$-D/$N^7$-$\beta$-D-ratio for **54** (**55**) by integration of HC(1') ($^1$H-NMR) (bottom).

The acetyl protecting groups from pyrimidine nucleoside **48** were removed by treatment with $NH_3$/MeOH (**Scheme 14**) in 91% yield. In the case of the homo-deoxycitidine derivative **50**, the acetyl protecting groups were selectively removed with 0.2 N NaOH at 0° in 25 min, directly

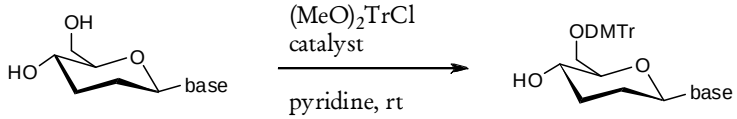
forming the $N^4$-protected precursor **58** necessary for oligonucleotide synthesis in a yield of 82%. $N^6$-benzoyl-homo-deoxyadenosine **59** was obtained under the same deprotection conditions applied for the preparation of **58**. The $\alpha$-D- and $\beta$-D-anomers of the adenine-nucleoside **59** were separated by recrystallisation from boiling MeOH, resulting in pure-$\beta$-D-homo-deoxyadenosine **59** in a 58% yield. After deprotection of the $N^9$-$\alpha$-D/$N^9$-$\beta$-D/$N^7$-$\beta$-D mixture of the homo-guanosine **54**$\beta$/**54**$\alpha$/**55**$\beta$ derivatives, the $N^7$-$\beta$-D-isomer was separated from $N^9$-$\alpha$-D/$N^9$-$\beta$-D-isomers by chromatography resulting in an anomeric mixture of **60**$\beta$/**60**$\alpha$ = 83/17.




**Scheme 14** Deprotection of the homo-DNA-nucleosides **48**, **50**, **53** and **54**.

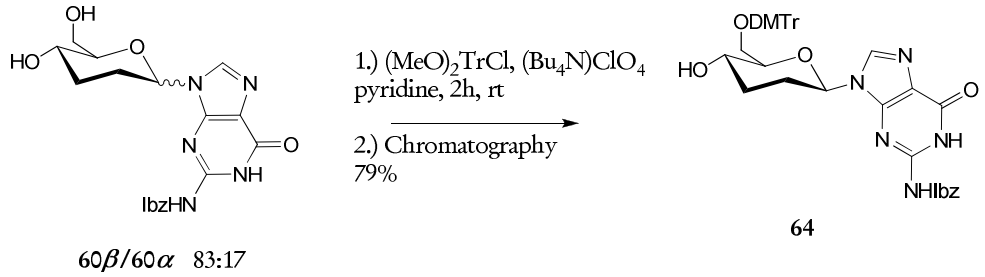
### 4.3.4 Synthesis of phosphoramidites of homo-DNA-nucleosides

The precursors **61**-**64** of the phosphoramidites **65**-**68** were prepared according to reported synthesis protocols.[79] Treatment of homo-uridine **57** with chloro(4,4'-dimethoxytriphenyl)methane ($(MeO)_2TrCl$) in pyridine with 4-(dimethylamino)pyridine (DMAP) as acylating catalyst and a tertiary amino base resulted selectively in the primary hydroxy-alkylated dimethoxytrityl derivative **61**. For the benzoylcytosine-, benzoyladenine- and isobutyric-guanosine derivatives **58**-**60**, tetrabutylammonium perchlorate ($(Bu_4N)ClO_4$) was used as acylating catalyst as described by *Beaucage* and coworkers,[80] because *Eschenmoser* and coworkers reported that DMAP only resulted in low yielding dimethoxytritylation (**Scheme 15**) in these cases.[32a] In the case of the guanosine derivative **60**, the dimethoxytritylether formation on the primary hydroxy group allowed a separation of the two anomer: **64**$\boldsymbol{\beta}$ was separated from **64**$\boldsymbol{\alpha}$ by chromatography on $SiO_2$.



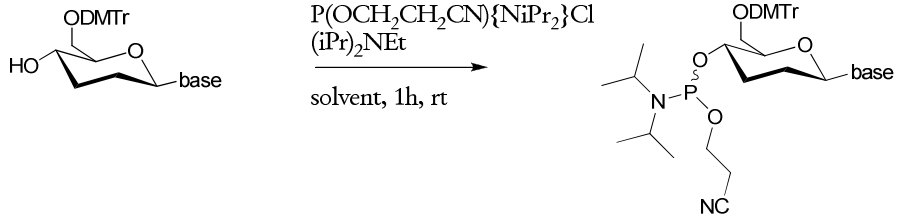

| Starting material | Nucleobase | Catalyst | Reaction time | Yield | product |
|---|---|---|---|---|---|
| **57** | uracil | DMAP/$Et_3N$ | 3.5 h | 75% | **61** |
| **58** | $Bz^4$cytosine | $(Bu_4N)ClO_4$ | 1 h | 81% | **62** |
| **59** | $Bz^6$adenine | $(Bu_4N)ClO_4$ | 1 h | 91% | **63** |

DMAP = 4-(dimethylamino)pyridine




**Scheme 15** Synthesis of homo-DNA-DMTr-derivatives **61-64** from 4',6'-diols **57-60**.

The dimethoxytrityl precursors **61-64** were converted to the (2-cyanoethyl)phosphoramidites **65-68** with chloro(2-cyanoethoxy)(diisopropylamino)phosphine and *Hünig*'s-base. The crude phosphoramidites **65-68** were, after aqueous workup and flash-chromatography on basic silica (addition of $Et_3N$), reprecipitated several times from rapidly stirred dichloromethane (resp. EtOAc)/hexane (to remove traces of $Et_3N$ that might inactivate the acidic activator used for the oligonucleotide-synthesis). Phosphoramidites **65-68** were obtained in 65-90% yield as 1:1 diastereomeric mixtures ($^1$H and $^{31}$P-NMR) (**Scheme 16**).




| Starting material | Nucleobase | Solvent | Yield | product |
|---|---|---|---|---|
| **61** | uracil | $CH_2Cl_2$ | 84% | **65** |
| **62** | Bz$^4$cytosine | THF | 87% | **66** |
| **63** | Bz$^6$adenine | THF | 78% | **67** |
| **64** | Ibz$^2$guanine | THF | 67% | **68** |

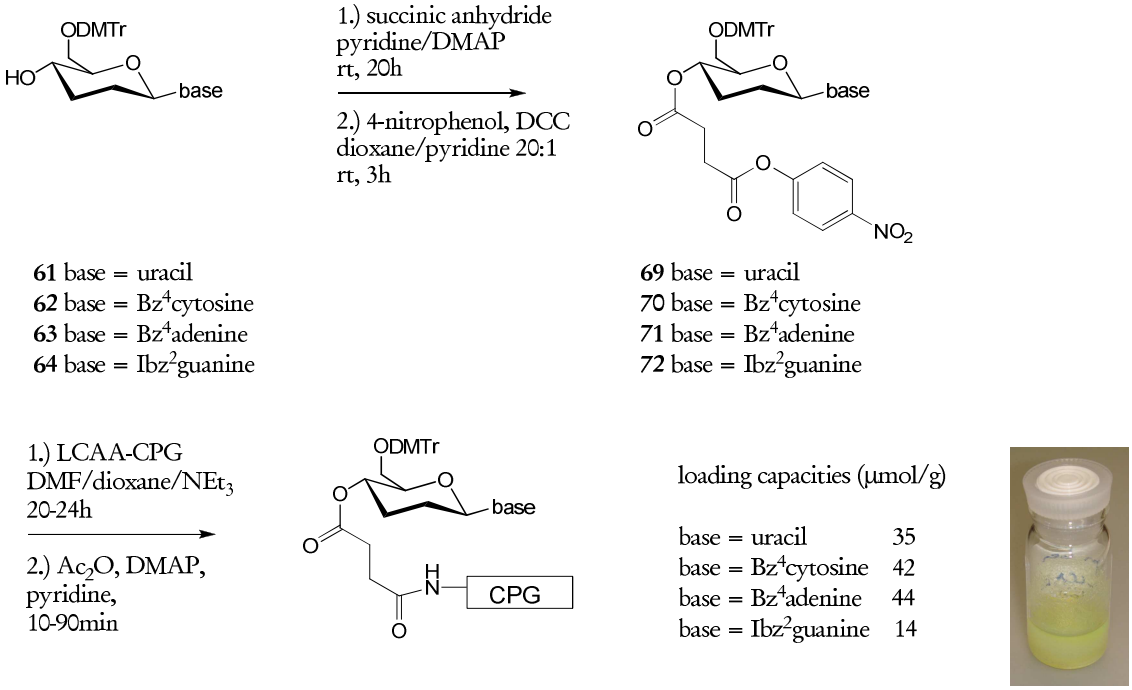
**Scheme 16** Preparation of phosphoramidites **65-68**.

### 4.3.5 Synthesis of controlled pore glass (CPG) derivatised homo-DNA-nucleosides

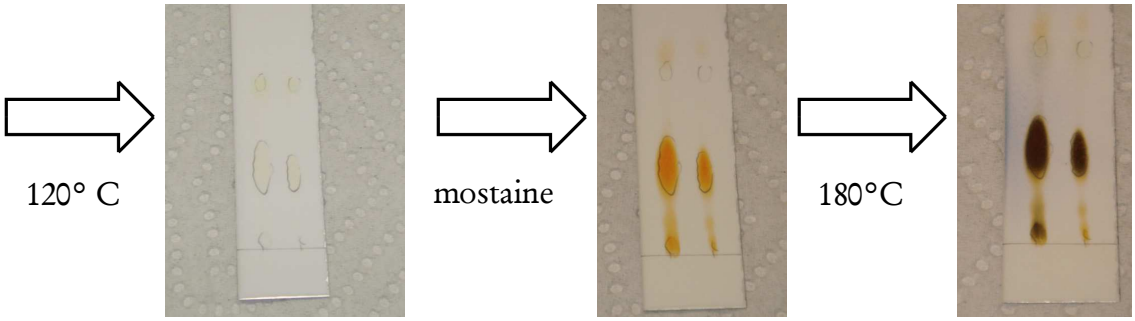
Activated esters **69**-**72** were prepared from the dimethoxytrityl derivatives **61**-**64** by an reaction with succinic anhydride in pyridine, followed by an esterification of the remaining carboxylic acid function with 4-nitrophenol in the presence of *N*,*N*'-dicyclohexylcarbodiimide (DCC) in good yields (**Scheme 17**). The 4-nitrophenylesters **69**-**72** were selectively detected on tlc plates by the observation that these compounds undergo a hydrolysis at 120°C, forming the yellow 4-nitrophenol. Spraying with the acidic mostain reagent (see Exp. Part) resulted in an immediate deprotection of the DMTr-protecting group and formation of the orange 4,4'-(dimethoxy)trityl-cation ($\lambda_{max}$. 498 nm) (**Scheme 17**, bottom). Another heating step to 180°C changed the color to brown, a color also observed for other sugar containing compounds with mostain reagent.

Activated esters **69**-**72** were immobilized on "controlled pore glass" (CPG) covered with alkylamino-groups. In first attempts, a 500 Å pore size, highly loaded (140 μmol/g) long chain alkylamine-CPG (LCAA-CPG) resin (*3-prime*) with a grain size of 75-125 μm was used. This LCAA-CPG had the disadvantage that it was necessary to perform filtration over P4-glas-filter funnels (pore size 10-16 μm), resulting in a slow filtration. This problem was circumvented by using a 500 Å pore size LCAA-CPG with a larger grain size of 125-175 μm (*SynGen*) and a lower loading capacity (60-80 μmol/g) making faster filtrations with P3-glass filter funnels (pore size 16-40 μm) possible. All reported loading capacities in Scheme 17 refer to the *SynGen*-LCAA-CPG with the larger particle size. The highly loaded LCAA-CPG (smaller particle size) was coated with higher loading capacities (typically values > 45 μmol/g were found for the *3-prime*-LCAA-CPG (data not shown)). The loading of the support was performed in a reaction of the activated esters **69**-**72** with a suspension of LCAA-CPG in DMF/dioxane/$Et_3N$ during 20-24 h at rt. During the addition of the solution containing the 4-nitrophenylesters **69**-**72**, the suspension immediately turned yellow, due to the formation of 4-nitrophenol. Capping the remaining free amino groups on the LCAA-CPG as amides with $Ac_2O$/DMAP in pyridine for 10-90 min resulted in the homo-

DNA-derivatised, capped LCAA-CPG-solid supports. If free primary amino groups were detected on the support by a ninhydrin tests according to a procedure described by *Kaiser* et al.[81] for the protein synthesis, another capping step with $Ac_2O$/DMAP in pyridine was performed. This step was repeated until the ninhydrin test showed negative results.




Ibz = isobutyric amide; DCC = dicyclohexylcarbodiimide; LCAA-CPG = long chain alkylamine-controlled pore glass




**Scheme 17** Preparation of polymer-bound protected homo-deoxyribonucleosides. Loading capacities (µmol/g) were obtained, as measured by photometric trityl assay (top) and selective detection of 4-nitrophenylesters (**69-72**) on tlc plates (bottom).

The loadings of the supports were determined by a photometric determination of the concentration of the 4,4'-(dimethoxy)trityl cation ($\lambda_{max.}$ 498 nm, $\varepsilon_{498}$ = 70000), produced from a defined aliquot of the derivatised support under acidic catalysis (0.1 M TsOH in $CH_3CN$ or 2% dichloroacetic acid in $CH_2Cl_2$) (**Fig. 32**).[79]

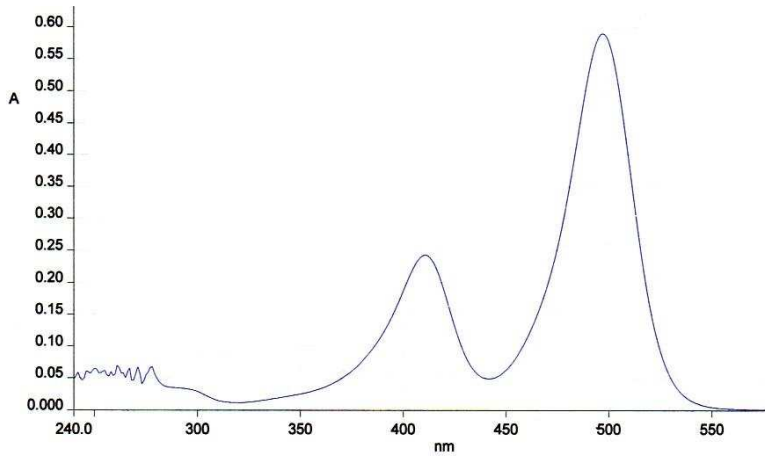
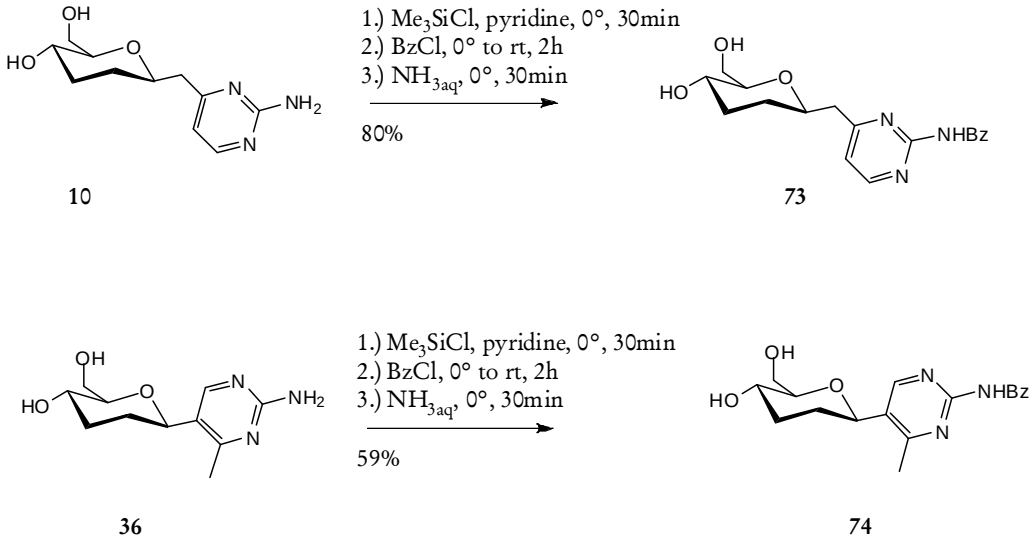



**Fig. 32** Example of a UV-spectrum of the 4,4'-dimethoxytrityl-cation obtained from deprotection of derivatised LCAA-CPG-support under acid catalysis, showing a strong absorption at 498 nm ($\varepsilon_{498}$ = 70000).

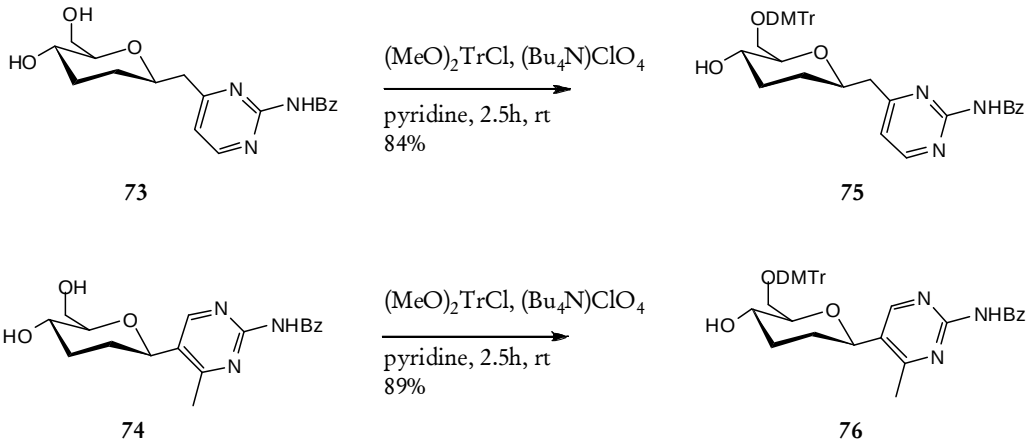
## 4.3.6 Synthesis of phosphoramidites of homo-DNA-*C*-nucleosides

The conversion of completely deprotected homo-DNA-*C*-alkyl- and homo-DNA-*C*-aryl-nucleosides **10** and **36** into the *N*-benzoyl-derivatives **73** and **74** was performed in a one pot reaction referring to known methods.[79] Silylation of the free OH-groups with $Me_3SiCl$ in pyridine at 0°, followed by benzoylation of the $NH_2$-functionality with BzCl and a deprotection of the bis-trimethylsilylethers with aq. $NH_3$, resulted in *N*-benzoylated homo-DNA-*C*-alkyl-nucleoside **73** and *N*-benzoylated homo-DNA-*C*-aryl-nucleoside **74** with yields of 80 and 59%, respectively (**Scheme 18**).



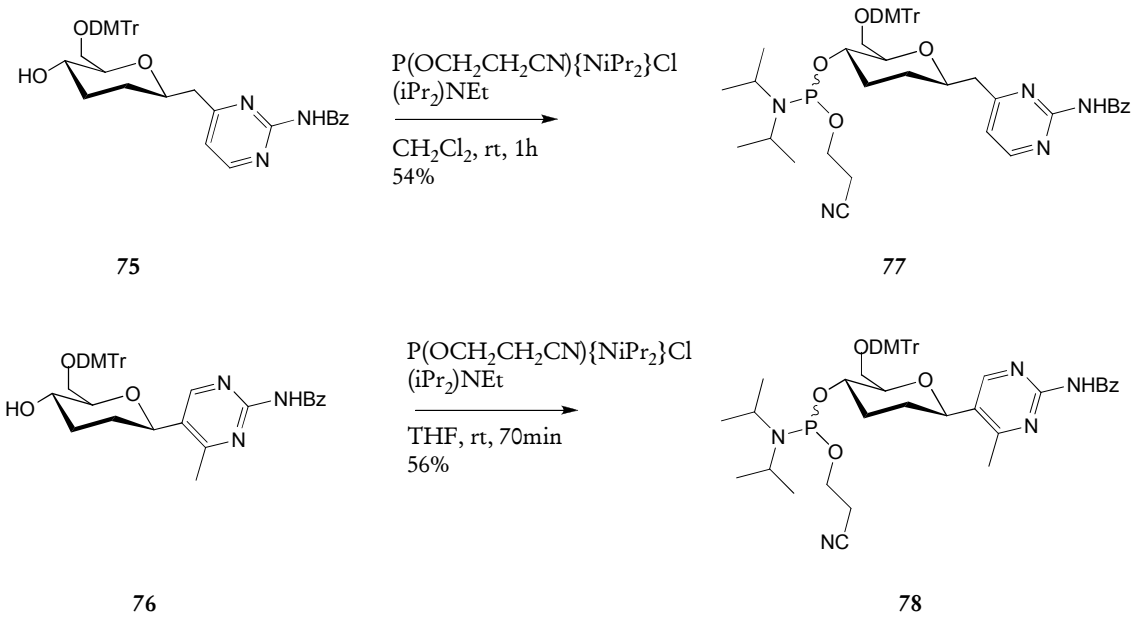

**Scheme 18** Synthesis of *N*-benzoyl-homo-DNA-*C*-alkyl- and *N*-benzoyl-homo-DNA-*C*-aryl-nucleosides **73** and **74**.

The nucleosides **75** and **76** were prepared according to reported synthetic protocols.[79] Treatment of homo-DNA-*C*-alkyl-nucleoside **73** with chloro(4,4'-dimethoxytriphenyl)methane ($(MeO)_2TrCl$) in pyridine with tetrabutylammonium perchlorate ($(Bu_4N)ClO_4$) as acylating catalyst resulted in selective alkylation of the primary hydroxy as dimethoxytrityl derivative **75**.[80] Attempts to apply 4-(dimethylamino)pyridine (DMAP) as acylating catalyst resulted in poor conversion in the case of homo-DNA-*C*-alkyl-nucleoside **73**, as it was already described by *Eschenmoser* and coworkers (**Scheme 19**) for other *N*-amide-protected homo-DNA-nucleosides, especially benzoylcytosine-, benzoyladenine- and isobutyric-guanosine-derivatives **58**-**60**.[32a] The homo-DNA-*C*-aryl-nucleoside **74** was converted to the dimethoxytrityl-derivative **76** under similar conditions as those applied to **73** with $(Bu_4N)ClO_4$ as acylating catalyst. Both conversions proceeded in yields of over 80%.




**Scheme 19** Synthesis of the 4,4'-dimethoxytrityl derivatives of homo-DNA-*C*-alkyl- and homo-DNA-*C*-aryl-nucleosides **75** and **76**.

The dimethoxytrityl precursors **75**, and **76** were converted to the (2-cyanoethyl)phosphoramidites **77**, and **78** with chloro(2-cyanoethoxy)(diisopropylamino)phosphine and *Hünig*'s-base. The crude phosphoramidites **77**, and **78** were, after aqueous workup and flash-chromatography on basic silica (addition of $Et_3N$), reprecipitated three times from rapidly stirred dichloromethane/hexane. (2-cyanoethyl)phosphoramidites. **77** and **78** were obtained in 54% yield as 1:1 diastereomeric mixtures (according to $^{1}H$- and $^{31}P$-NMR) (**Scheme 20**).




**Scheme 20** Conversion of dimethoxytrityl-derivatives **75**, and **76** to (2-cyanoethyl)phosphoramidites **77**, and **78**.

## 4.3.7 Oligonucleotide synthesis

Oligonucleotides in amounts >3 μmol were prepared by manual solid-phase synthesis according to reported procedures[79] with application of (2-cyanoethyl)phosphoramidites as monomer units and 5-(ethylthio)-1*H*-tetrazole as activator. Small scale oligonucleotide preparations <1 μmol (typically 0.2 μmol or 0.4 μmol) for characterisation, UV-melting curve studies, CD and neg. ESI- resp. MALDI-TOF-MS were performed with (2-cyanoethyl)phosphoramidites on a DNA-synthesizer (*Expedite* NAS 8905, *Applied Biosystems*), using a protocol developed for DNA synthesis and successfully applied for homo-DNA synthesis.[32a] Our protocol varied from the standard specification according to *Applied Biosystems* in the coupling times, which were increased for some couplings (see Experimental part), the activator (the more acidic and more potent 5-(ethyltio)-1*H*-tetrazol instead of 1*H*-tetrazol was

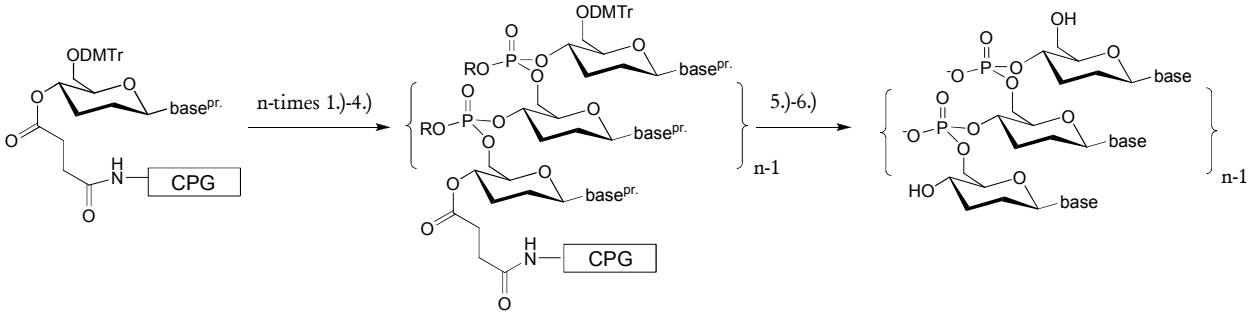
used) and in later synthesis the concentration of (2-cyanoethyl)phosphoramidites in later synthesis (0.05 M compared to 0.1 M), allowing a reduction of the (2-cyanoethyl)phosphoramidite used for purging the lines (**Scheme 21**). The chain construction cycle consisted of four steps:

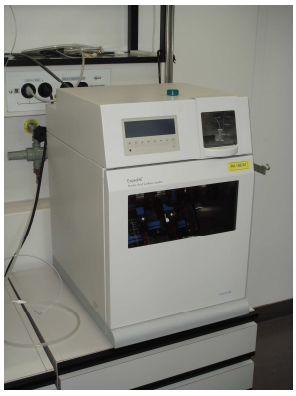
1.) Acidic cleavage of the DMTr-protecting group and release of the 6'-OH-group of the support-bound nucleoside (respectively oligonucleotide).

2.) Coupling with the 5-(ethylthio)-1*H*-tetrazol activated (2-cyanoethyl)phosphoramidite.

3.) Capping of uncoupled 6'-OH-groups with $Ac_2O$.

4.) Oxidation of the phosphite to the phosphate with an aqueous $I_2$-solution.



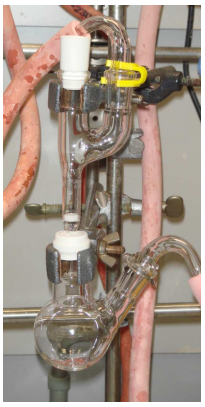
Expedite NAS 8905, DNA synthesizer

chain elongation

1.) deprotection: 3% trichloroacetic acid in $CH_2Cl_2$,40s , wash
2.) coupling: 0.1M phosphoramidite (10 eq) in $CH_3CN$,
0.5M 5-(ethylthio)-1*H*-tetrazol (30 eq) in $CH_3CN$, 1.5 to 5 min, wash
3.) capping: 7% 1-methylimidazole in
THF/ 2,6-dimethylpyridine/ $Ac_2O$ 15:1:1, 30s, wash
4.) oxidation: 0.1 M $I_2$ in THF/pyridine/$H_2O$ 4:1:0.1, 50s wash

release of the support and deprotection

5.) deprotection: 3% trichloroacetic acid in $CH_2Cl_2$, 40s, wash
6.) conc. $NH_3$-solution, 20h, 55°.



manuall oligonucleotide synthesis

chain elongation

1.) deprotection: 2% dichloroacetic acid in $CH_2Cl_2$, 2min, wash
2.) coupling: phosphoramidite (8 eq.), 30 eq 5-(ethylthio)-1*H*-tetrazol,
$CH_3CN$, 1h, wash
3.) capping: DMAP, $Ac_2O$, 2,6-dimethylpyridine, THF, 5 min, wash
4.) oxidation: $I_2$, THF/$H_2O$/2,6-dimethylpyridine 2:2:1, 1 min, wash

release of the support and deprotection

5.) deprotection: 2% dichloroacetic acid in $CH_2Cl_2$, 2min, wash
6.) conc. $NH_3$-solution, 20h, 55°.

**Scheme 21** Oligonucleotide synthesis cycle for *Expedite* NAS 8905 DNA synthesizer (*Applied Biosystems*) and manual oligonucleotide synthesis (right), *Expedite* NAS 8905 DNA synthesizer (*Applied Biosystems*) (middle, left), and apparatus constructed for manual oligonucleotide synthesis (bottom, left).

Coupling yields were determined according to the photometric dimethoxytrityl cation assay (**Fig. 32**) and were typically >97% for the automated homo-DNA synthesis. In the manual homo-DNA synthesis a coupling yield of >98% was observed. After finishing chain elongation, the deprotection process was initiated by cleavage of the 6'-terminal dimethoxytrityl group, followed by a release from the support and cleavage of all nucleobases- and phosphate-protecting groups with concentrated $NH_3$-solution at 55° C for 20 h resulting in homo-DNA-oligodeoxyribonucleotides as crude materials. The purity of these homo-DNA-oligonucleotides was determined by reversed-phase (RP) $C_{18}$-HPLC ($C_{18}$-*Waters* Spherisorb ODS2, 5 μm, 250x4.6 mm) using a linear gradient of $CH_3CN$ in 0.1 M aqueous $(Et_3NH)OAc$, pH 7.0 or *DEAE*-ion exchange (IE) HPLC (*Nucleogen-DEAE* 60-7, *Macherey-Nagel*) with a linear KCl-gradient in 20 mM $KH_2PO_4$, pH 6.0, in $H_2O/CH_3CN$ 4:1. If necessary, oligonucleotides were purified by an identical HPLC-method. It was shown by MALDI-TOF-MS of several homo-DNA-oligonucleotides prepared with poor coupling yields (about 80%) that $C_{18}$-RP- and DEAE-ion exchange-HPLC allow a separation of mixtures containing homo-DNA-oligonucleotides, which only differentiate in chain length in a single nucleotide. Pure homo-DNA oligonucleotide sequences were purified by precipitation from 70% EtOH / 30 % 0.3 M Na-acetate in $H_2O$, pH = 4.8.

First attempts to analyze homo-DNA-oligonucleotides were performed with negative electrospray ionisation (ESI)-MS, producing good results for the monoanionic dinucleotide ddGlc[$U_2$]. However, polyanionic homo-DNA species with the general formula ddGlc[$A_aB_bC_cD_dE_e$], $\Sigma_{a,b,c,d,e}>2$ gave results that were difficult to interpretate. The homo-DNA-hexamer ddGlc[$(UA)_3$], for example, showed numerous signals in the neg. ESI-MS, of which the

dianionic species $[m+3H]^{2-}$, $[m+2H+Na]^{2-}$, $[m+2H+K]^{2-}$, $[m+H+2Na]^{2-}$ were the most intensive ones (**Fig. 33**, bottom).

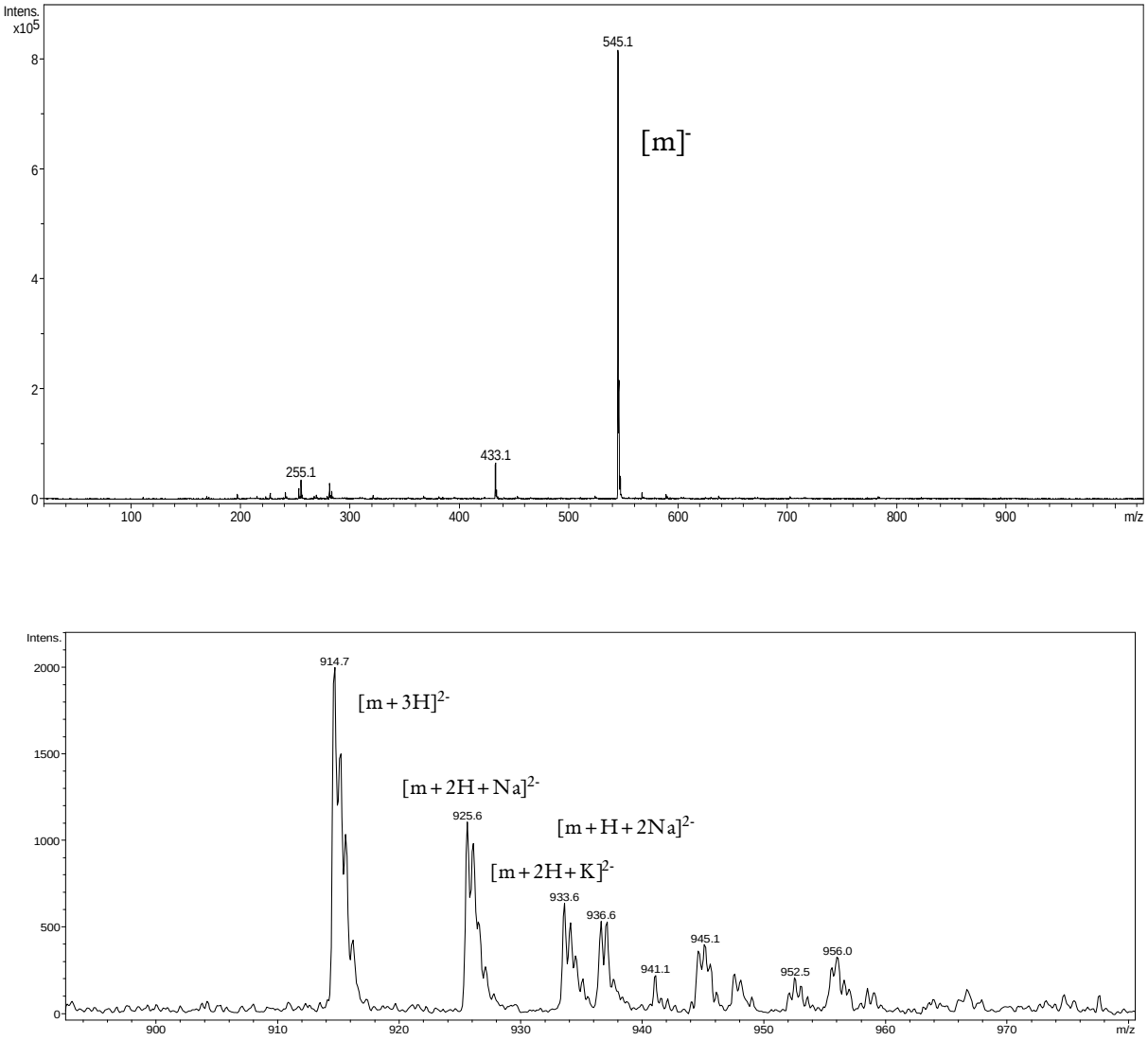



**Fig. 33** Neg. ESI-MS of the dimer ddGlc[$U_2$] (top) and the hexamer ddGlc[$(UA)_3$] (bottom).

For homo-DNA-oligonucleotides with the general formula ddGlc[$A_aB_bC_cD_dE_e$], $\Sigma_{a,b,c,d,e}>2$, the method of choice for analysis was matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) MS with a 6-aza-2-thiothymine (ATT) matrix.[82] MALDI-TOF measurement under these conditions resulted, also for longer polyanionic homo-DNA sequences, in a main peak corresponding to the molecular monoanion of the oligomer (**Fig. 34**).

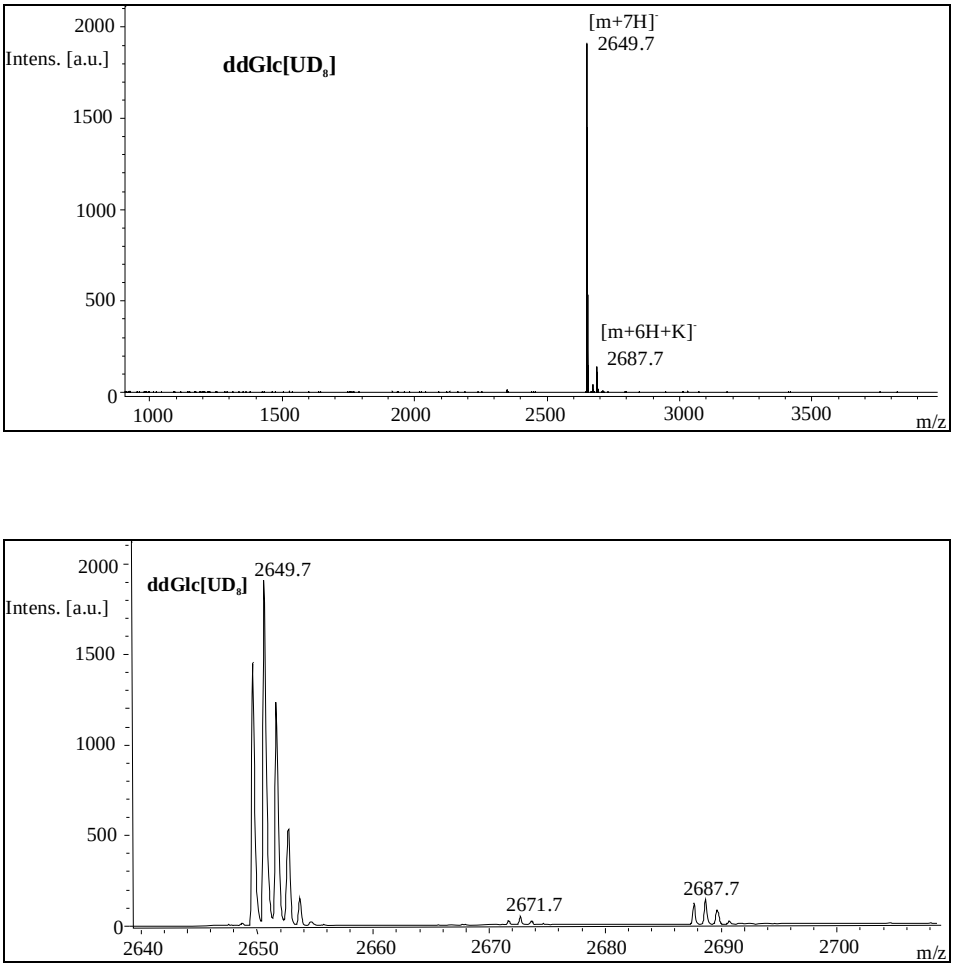



**Fig. 34** MALDI-TOF MS (matrix: 6-aza-2-thiothymine) of the 9-mer ddGlc[$UD_8$]. The main peak corresponds to the molecular monoanion of the corresponding oligomer.

| Sequence | Manual (m) or automatic (a) oligonucleotide-synthesis | Scale [µmol] | Coupling time | $C_{18}$-RP-HPLC (buffer A/B) or Nucleogen DEAE 60-7-IE-HPLC (buffer C/D), retention time | MS analysis (method, calc., found) |
|---|---|---|---|---|---|
| ddGlc[$U_2$] | m | 3.5 | 60 min | 0-20% B in 17min, $t_R$ = 13.9 min | Neg. HR-ESI calc. $[m]^-$ 545.1285, found 545.1291 |
| ddGlc[$U_2$] | a | 0.2 | 3 min 30 s | 0-20% B in 17min, $t_R$ = 13.9 min | Neg. ESI, calc. $[m]^-$ 545.1, found 545.1 |
| ddGlc[$U_3$] | a | 0.2 | 3 min 30 s | 0-30% B in 27 min, $t_R$ = 15.1 min | |
| ddGlc[$U_4$] | a | 0.2 | 1 min 30 s | 0-30% B in 27 min, $t_R$ = 15.2 min | |
| ddGlc [$U_6$] | a | 0.2 | 3 min 30 s | 0-50% B in 12 min, $t_R$ = 8.7 min | |
| ddGlc[$^{4'}(UA)_3{}^{6'}$] | a | 0.2 | 3 min 30 s | 0-30% B in 27min, $t_R$ = 18.0 min | Neg. ESI, calc. $[m+3H]^{2-}$ 1829.4, found 1829.4 // calc. $[m+2H+Na]^{2-}$ 1851.4, found 1851.2 // $[m+2H+K]^{2-}$ 1867.3, found 1867.2 |
| ddGlc[$^{4'}(UA)_4{}^{6'}$] | a | 0.2 | 3 min 30 s | 0-30% B in 25 min, $t_R$ = 18.5 min | Neg. ESI, calc. $[m+5H]^{2-}$ 2460.5, found 2461.0 //calc. $[m+4H+Na]^{2-}$ 2482.5, found 2482.3 // calc. $[m+3H+2Na]^{2-}$ 2504.5, found 2505.2 |
| ddGlc[$C_7$] | a | 0.2 | 1 min 30 s | 0-30% B in 25 min, $t_R$ = 16.4 min | MALDI-TOF (Matrix ATT), calc. $[m+5H]^-$ 2058.4, found 2058.7 |
| ddGlc[$G_7$] | a | 0.2 | 3 min 30 s | | MALDI-TOF (Matrix ATT), calc. $[m+5H]^-$ 2338.5, found 2337.8 |
| ddGlc[$^{4'}UD_8{}^{6'}$] | a | 0.4 | 1 min 30 s | 15-75% D in 30 min, $t_R$ = 20.8 min | MALDI-TOF (Matrix ATT), calc. $[m+7H]^-$ 2649.7, found 2649.7 |

| | | | | | |
|---|---|---|---|---|---|
| ddGlc[$^{4'}$UAUD(UA)$_2$$^{6'}$] | a | 0.4 | 1 min 30 s | 15-75% D in 30 min, $t_R$ = 15.5 min | MALDI-TOF (Matrix ATT), calc. [m+6H]$^-$ 2435.5, found 2435.5 |
| ddGlc[$^{4'}$UD(UA)$_3$$^{6'}$] | a | 0.4 | 1 min 30 s | 15-75% D in 30 min, $t_R$ = 14.6 min | MALDI-TOF (Matrix ATT), calc. [m+6H]$^-$ 2435.5, found 2435.3 |
| ddGlc[$^{4'}$(UA)$_2$UD(UA)$_2$$^{6'}$] | a | 0.4 | 1 min 30 s | 15-75% D in 30 min, $t_R$ = 17.6 min | MALDI-TOF (Matrix ATT), calc. [m+8H]$^-$ 3066.6, found 3066.5 |
| ddGlc[$^{4'}$(UA)$_3$UD(UA)$_3$$^{6'}$] | a | 0.4 | 1 min 30 s | 15-75% D in 30 min, $t_R$ = 24.4 min | MALDI-TOF (Matrix ATT), calc. [m+12H]$^-$ 4328.9, found 4329.2 |
| ddGlc[U$_8$] | a | 0.4 | 1 min 30 s | 15-75% D in 30 min, $t_R$ = 9.7 min | MALDI-TOF (Matrix ATT), calc. [m+5H]$^-$ 2369.4, found 2369.2 |
| ddGlc[$^{4'}$(UA)$_5$$^{6'}$] | a | 0.4 | 1 min 30 s | 15-75% D in 30 min, $t_R$ = 20.2 min | MALDI-TOF (Matrix ATT), calc. [m+8H]$^-$ 3092.6, found 3092.6 |
| ddGlc[$^{4'}$AADA$_5$$^{6'}$] | a | 0.4 | 1 min 30 s | 15-75% D in 30 min, $t_R$ = 23.1 min | MALDI-TOF (Matrix ATT), calc. [m+6H]$^-$ 2527.6, found 2527.5 |
| ddGlc[A$_8$] | a | 0.4 | 1 min 30 s | 15-75% D in 30 min, $t_R$ = 26.4 min | MALDI-TOF (Matrix ATT), calc. [m+6H]$^-$ 2553.6, found 2553.5 |
| ddGlc[$^{4'}$UCACAUAUG$^{6'}$] | a | 0.4 | 1 min 30 s | | |
| ddGlc[$^{4'}$UCACCUAUG$^{6'}$] | a | 0.4 | 1 min 30 s | | |
| ddGlc[$^{4'}$UCACGUAUG$^{6'}$] | a | 0.4 | 1 min 30 s | | |
| ddGlc[$^{4'}$UCACUUAUG$^{6'}$] | a | 0.4 | 1 min 30 s | | |
| ddGlc[$^{4'}$CAUADGUGA$^{6'}$] | a | 0.4 | 1 min 30 s | | |
| ddGlc[$^{4'}$CAUAAGUGA$^{6'}$] | a | 0.4 | 1 min 30 s | | |

**A =** 0.1 M $(HNEt_3)OAc$ in $H_2O$, pH 7.0

**B =** $CH_3CN$

**C =** 20 mM $KH_2PO_4$ in $H_2O/CH_3CN$ 4:1, pH 6.0

**D =** 1 M KCl, 20 mM $KH_2PO_4$ in $H_2O/CH_3CN$ 4:1, pH 6.0

## *4.4 Base pairing studies*

In order to evaluate the influence of exchanging a homo-DNA-adenine nucleotide against the corresponding 2,4-diaminopyrimidine nucleotide both self-complementary and not self-complementary homo-DNA sequences were investigated (**Fig. 35**).

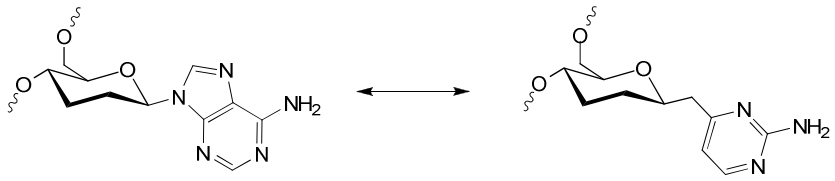


**Fig. 35** homo-DNA adenine nucleotide (left) and *C*-analoga of a homo-DNA 2,4-diaminopyrimidine nucleotide (right).

Within the studies of self-complementary homo-DNA sequences three questions were assessed:

1.) In homo-DNA sequences with the general formula ddGlc[$(AU)_n$] the influence of the adenine – 2,4-diaminopyrimidine exchange in *Watson-Crick* A-U base pairings was evaluated.

2.) In self-complementary poly-adenine homo-DNA oligonucleotides the influence of adenine – 2,4-diaminopyrimidine exchanges on reverse *Hoogsteen* A-A base pairings was evaluated.

3.) In non self-complementary homo-DNA sequences with the general formula ddGlc[$^{4'}$CAUA-X-GUGA$^{6'}$] and ddGlc[$^{4'}$UCAC-Y-UAUG$^{6'}$] the influence of an adenine – 2,4-diaminopyrimdine exchange in position X with Y being uracil, adenine, cytosine and guanine was evaluated.

### 4.4.1 Self-complementary sequences

#### 4.4.1.1 Homo-DNA sequences with the general formula ddGlc[$(AU)_n$]

Because of the reported strong reverse-*Hoogsten*-base-pairing of ddGlc[$A_n$], with n>4 (see 3.2.1) which could not be broken up by the addition of ddGlc[$T_n$], with n>4 as it was reported by *Eschenmoser* and coworkers,[32c] it had to be expected that, as the *Watson-Crick* A-T base-pairing, also the *Watson-Crick* A-U-base-pairing in the homo-DNA-system would be detectable in self-complementary sequences like ddGlc[$(AU)_n$], but not by addition of ddGlc[$U_n$] to ddGlc[$A_n$]. For that reason was decided to make a comparison of a *Watson-Crick* adenine-uracil base pairing with a diaminopyrimidine-uracil base pairing in self-complementary oligonucleotides with the general formula ddGlc[$(AU)_n$] in which a single adenine nucleotide was replaced by a diaminopyrimidine nucleotide. It was shown, as it will be reported in the next few paragraphs, that the influence of a single adenine-diaminopyrimidine exchange was especially influenced by two parameters:

1.) the position of the modification: A replacement of an adenine nucleotide in a central position had a more disturbing influence on the duplex stability (expressed by $T_m$) compared to an adenine-dimaminopyrimidine exchange near the 4'-end of a homo-DNA-oligonucleotide.

2.) for central adenine-diaminopyrimidine exchanges, a strong dependency of the melting behavior from the chain length was observed: The decamer ddGlc[$(UA)_2UD(UA)_2$] showed a decrease of $T_m$ with increasing oligonucleotide concentration in solution. For the 14-mer ddGlc[$(UA)_3UD(UA)_3$] a decreasing $T_m$ with increasing concentration was found for concentrations >6 μM. For concentrations <6 μM, no concentration dependency of the $T_m$ was observed in the 14-mer ddGlc[$(UA)_3UD(UA)_3$] indicating intramolecular interaction.

From the self-complementary (referring to antiparallel strand orientation) ddGlc[$(UA)_n$] with n =3 and 4 melting curves were determined in 0.15 M NaCl, 0.01 M TrisHCl buffer, pH 7.0 by temperature dependant UV- and CD-spectroscopy of solutions with different oligonucleotide concentrations. The oligonucleotide concentrations in solution were determined by a comparison

of the calculated sum of the extinction coefficients of all mononucleosides at $\lambda$ = 260 nm with the measured optical density at this wavelength at a temperature *T* higher than the experimentally determined melting point for this oligonucleotide. $T_m$ values were determined according to values obtained from UV-melting curves (if at the $\lambda_{max}$ value a strong CD signal was observed, $T_m$ values were determined according to mean values obtained from UV- and CD-melting curves). *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) were determined according to described methods[83] by a linear correlation of the reciprocally $T_m$-values to the logarithm of the oligonucleotide concentration (see experimental part). An indication of the errors of the data was derived from the $R^2$-factors of this linear regression (**Fig. 36**, **Fig. 37**).

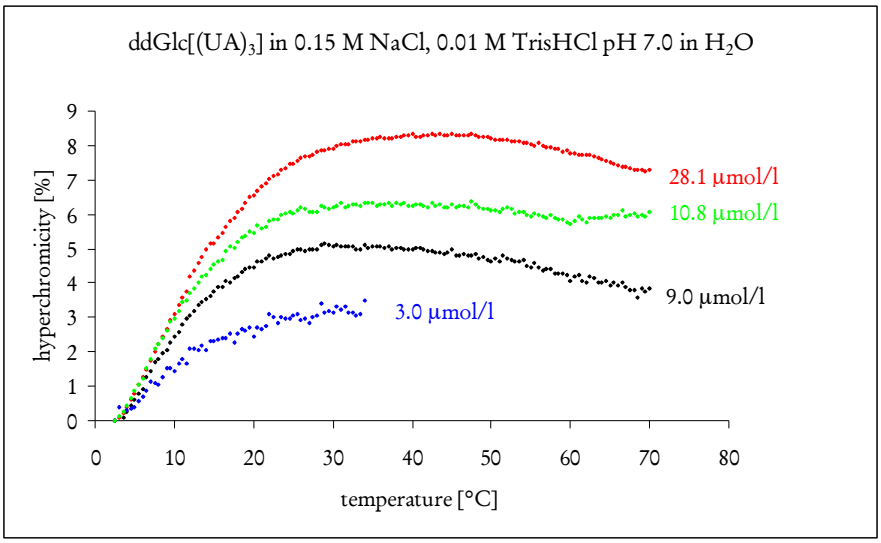
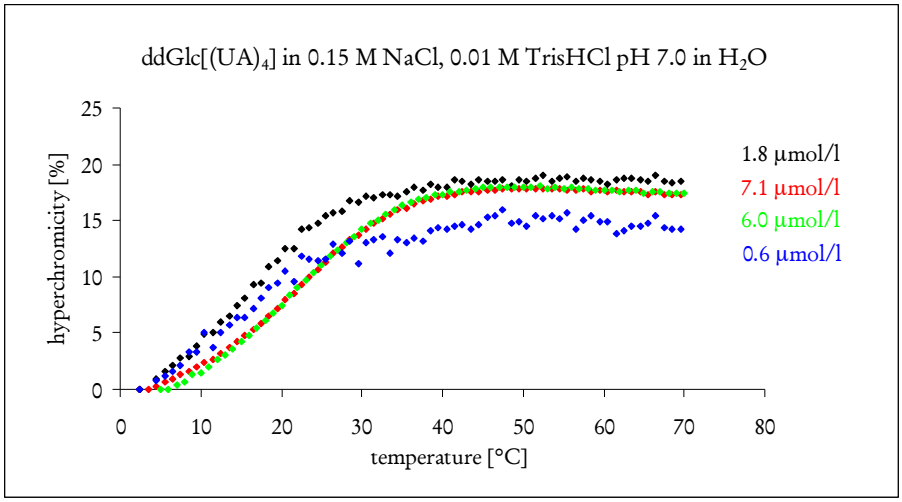


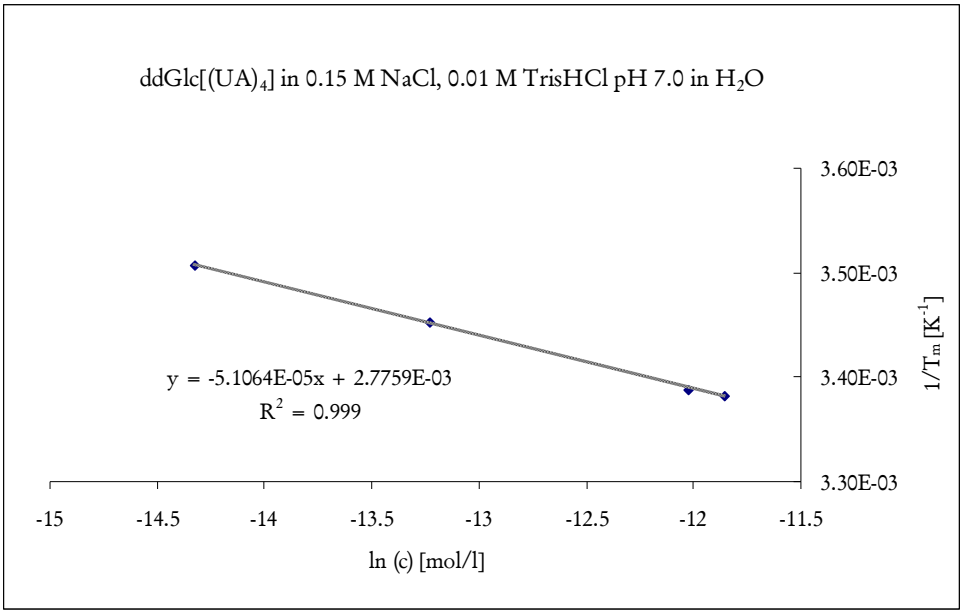

**Fig. 36** UV-melting curve of the hexanucleotide ddGlc[$(UA)_3$] in 0.15 M NaCl 0.01 M TrisHCl, pH 7.0. The increasing hyperchromicity values with increasing oligonucleotid concentration indicate that the hexamer ddGlc[$(UA)_3$] was already partially melted at the lowest measured temperature ($t$ = 2.5 °C).

In the temperature dependent UV-melting curve of the hexamer ddGlc[$(UA)_3$] increasing hyperchromicity values for increasing oligonucleotide concentratrations were detected. The 3.0 µM solution showed a hyperchromicity of 3 % (**Fig. 36**, blue melting curve), this value was

increased to 4.5 % for the 9.0 µM oligonucleotide solution (**Fig. 36**, black melting curve), 6 % for the 10.8 µM solution (**Fig. 36**, green melting curve) and reached 7.5 % for the highest measured 28.1 µM solution (**Fig. 36**, red melting curve), indicating that even with the 28.1 µM solution the homo-DNA-oligonucleotide is partially melted at $t$ = 2.5 °C, the lowest measured temperature (water based temperature control unit). This interpretation was confirmed by the observation that for the octanucleotide ddGlc[$(UA)_4$] in a concentration range between 0.6 µM and 7.1 µM constant hyperchromicity values of about 17 % were obtained (**Fig. 37**, top). For the hexamer ddGlc[$(UA)_3$] no calculation of *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) was performed, because the prerequisite for this determination is the observation of the completely unmelted duplex (method see[83b,84]).




| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | Hyperchromicity (wavelength) [%] |
|---|---|---|---|
| ddGlc[$(UA)_4$] | 0.6 | 12.0 | 16 (260 nm) |
| ddGlc[$(UA)_4$] | 1.8 | 16.5 | 18 (260 nm) |
| ddGlc[$(UA)_4$] | 6.0 | 22.0 | 17 (260 nm) |
| ddGlc[$(UA)_4$] | 7.1 | 22.5 | 17 (260 nm) |



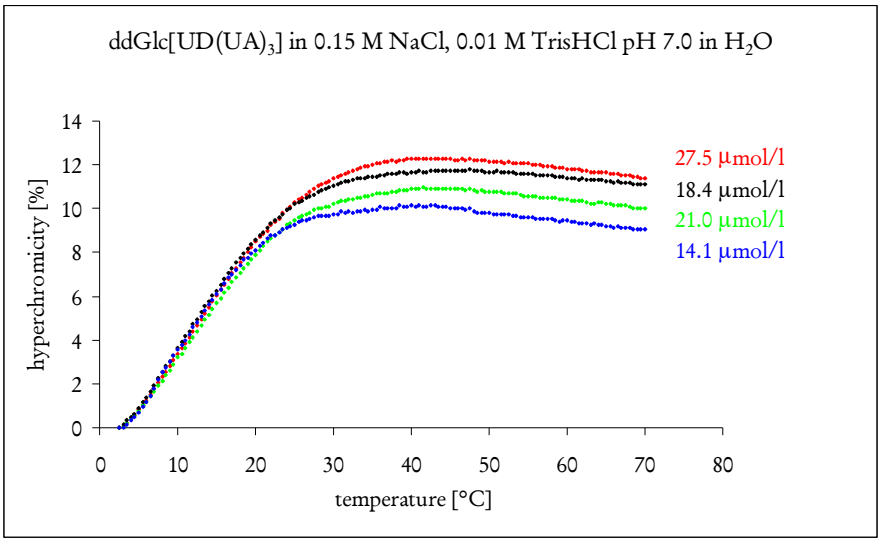

| **Oligonucleotide** | ***ΔH* [kJ/mol]** | ***ΔS* [J/(mol*K)]** | ***ΔG* (T=25 °C) [kJ/mol]** |
|---|---|---|---|
| ddGlc[$(UA)_4$] | -163 | -452 | -28.1 |

**Fig. 37** UV-melting curves of different oligonucleotide concentrations of the octanucleotide ddGlc[$(UA)_4$] in 0.15 M NaCl 0.01 M TrisHCl, pH 7.0 (top), determined $T_m$ values (average values obtained from UV- and CD-melting (data not shown)), hyperchromicities (middle), and linear regression $1/T_m$ vs. ln(c) resulting in $\Delta H$ and $\Delta S$ values (bottom).

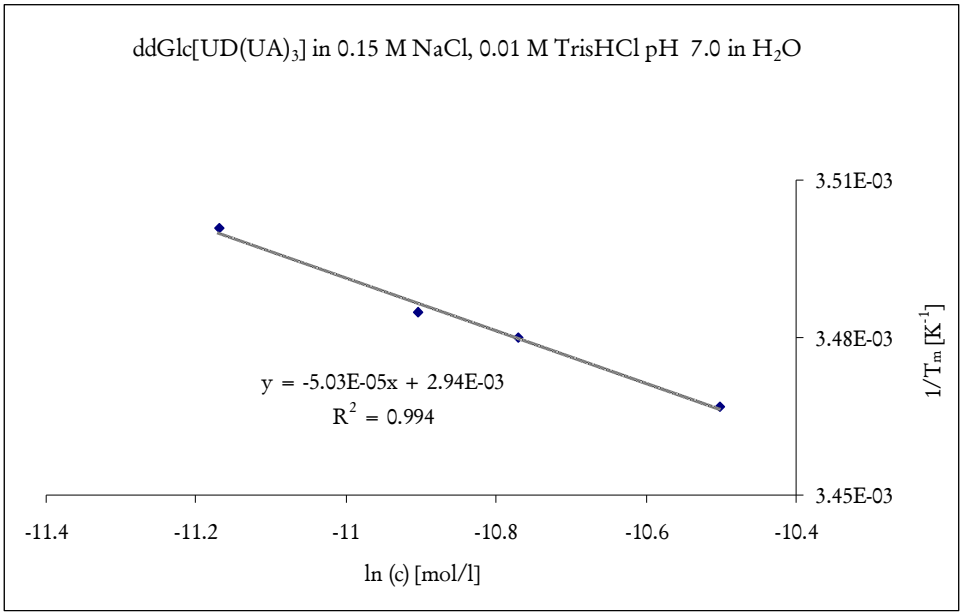
For the octamer ddGlc[$(UA)_4$], a sigmoid melting curve was obtained with constant hyperchromicity values (of about 17 %) for an oligonucleotide concentration between 0.6 µM and 7.1 µM (**Fig. 37**, middle). As expected for an intermolecular interaction, the $T_m$ values were increased with increasing oligonucleotide concentrations in solution. The $R^2$-value of 0.999 of the linear regression $1/T_m$ vs. ln(c) indicated a good fitting of the data and the determination of the *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$), resulted in $\Delta H$ = -163 kJ/mol and $\Delta S$ = -452 J/(mol*K) (**Fig. 37**).

In the self-complementary octamer ddGlc [UD$(UA)_3$], a sigmoid melting curve was obtained with constant hyperchromicity values (of 10-11 %) for an oligonucleotide concentrations between 27.5 µM and 14.1 µM. With lower oligonucleotide concentrations than 14.1 µM, the

hyperchromicity values were decreased, indicating a partially melted duplex at the initial temperature 2.5 °C (data not shown). As expected for intermolecular duplex formation the $T_m$ values were increased with increasing oligonucleotide concentration in solution. The $R^2$-value of 0.994 of the linear regression $1/T_m$ vs. ln(c) indicated a good fitting of the data and the determination of the *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$), resulted in $\Delta H$ = -165 kJ/mol and $\Delta S$ = -485 J/(mol*K) (**Fig. 38**).




| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | Hyperchromicity (wavelength) [%] |
|---|---|---|---|
| ddGlc[UD(UA)$_3$] | 14.1 | 12.5 | 9 (260 nm) |
| ddGlc[UD(UA)$_3$] | 18.4 | 13.8 | 10 (260 nm) |
| ddGlc[UD(UA)$_3$] | 21.0 | 14.2 | 11 (260 nm) |
| ddGlc[UD(UA)$_3$] | 27.5 | 15.3 | 11 (260 nm) |



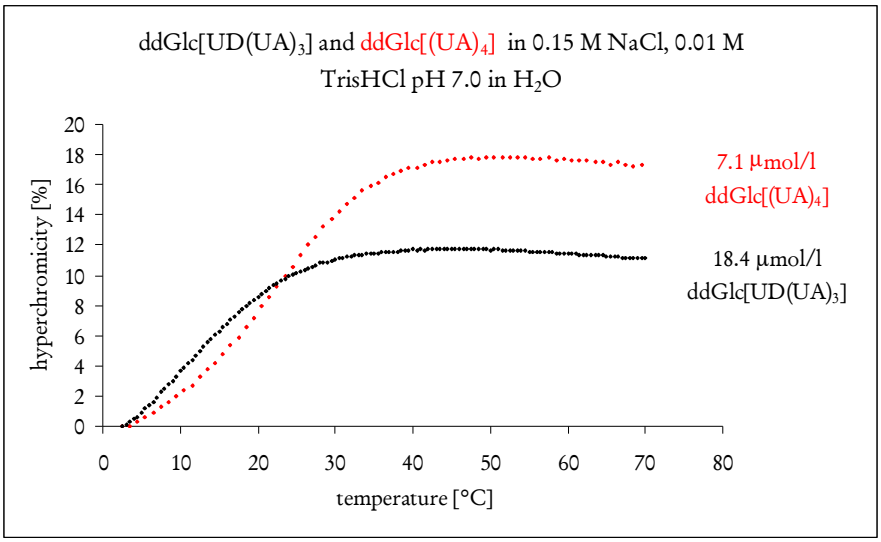

| Oligonucleotide | *ΔH* [kJ/mol] | *ΔS* [J/(mol*K)] | *ΔG* (T=25 °C) [kJ/mol] |
|---|---|---|---|
| ddGlc[UD$(UA)_3$] | -165 | -485 | -20.5 |

**Fig. 38** UV-melting curves of different oligonucleotide concentrations of the octanucleotide ddGlc[UD$(UA)_3$] in 0.15 M NaCl, 0.01 M TrisHCl, pH 7.0 (top), determined $T_m$ values (values obtained from UV- melting) hyperchromicities (middle), and linear regression $1/T_m$ vs. ln(c) resulting in *Van't Hoff*-pairing enthalpies (*ΔH*) and –entropies (*ΔS*) (bottom).

The comparison of the data obtained from the octamers ddGlc[$(UA)_4$] and ddGlc[UD$(UA)_3$] showed the following effects (**Fig. 39**): A decrease of the hyperchromicity values from about 17 % for ddGlc[$(UA)_4$] to 10 % for ddGlc[UD$(UA)_4$] was observed. The exchange of two *Watson-Crick*-adenine-uracil base pairs by two diaminopyrimidine-uracil base pairs, resulted in a weakening of the duplex stability at a concentration of 10 µM from 24.1 °C for ddGlc[$(UA)_4$] to 11.0 °C for ddGlc[UD$(UA)_3$] (calculated values according to the linear regression $1/T_m$ vs. ln (c) made for both oligonucleotides, for details see experimental part). This value corresponded to a weakening of duplex stability expressed by $T_m$ of 6.5 °C per exchange of a single Watson-Crick adenine-uracil vs. a diaminopyrimidine-uracil base pair, under the assumption that also ddGlc[UD$(UA)_3$] forms antiparallel oriented *Watson-Crick* base pairs, as it was shown for the dodecamer ddGlc[$(UA)_6$].[32]

Although no direct $T_m$ comparison between the octamer ddGlc [UD(UA)$_3$] and the hexamer ddGlc[(UA)$_3$] was accessible, due to the difficulty to observe the fully unmelted state in the hexamer ddGlc[(UA)$_3$], it is clear that the duplex stability is higher in the octamer ddGlc[UD(UA)$_3$], clearly indicating a diaminopyrimidine-uracil base pairing.

DdGlc[UD(UA)$_3$] showed a broader melting region compared to ddGlc[(UA)$_4$]. An evaluation of melting data obtained from melting curves of different oligonucleotide concentrations of both oligonucleotides showed that the lower duplex stability of ddGlc[UD(UA)$_3$] compared to ddGlc[(UA)$_4$] is only due to entropic reasons, but not to enthalpic reasons (**Fig. 39**). The replacement of two adenine-uracil *Watson-Crick* base pair against two diaminopyrimidine-uracil base pairs does not enthalpically destabilize the octamer ddGlc[UD(UA)$_3$] compared to ddGlc[(UA)$_4$].




| **Oligonucleotide** | ***ΔH* [kJ/mol]** | ***ΔS* [J/(mol*K)]** | ***ΔG* (T=25 °C) [kJ/mol]** |
|---|---|---|---|
| ddGlc[UD(UA)$_3$] | -165 | -485 | -20.5 |
| ddGlc[(UA)$_4$] | -163 | -452 | -28.1 |

**Fig. 39** UV-melting curves of the octanucleotides ddGlc[(UA)$_4$] and ddGlc[UD(UA)$_3$]in 0.15 M NaCl 0.01 M TrisHCl, pH 7.0.

It is tempting to speculate that this entropic destabilization is due to an increased flexibility of the nucleobase in the homo-DNA diaminopyrimidine nucleoside compared to the corresponding adenine nucleoside (**Fig. 40**).

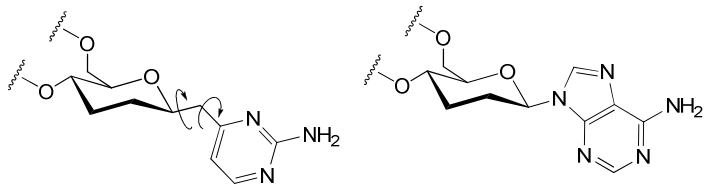


**Fig. 40** Additional degrees of freedom of a *C*-analoga of a 2,4-diaminopyrimdine homo-DNA-nucleotide (left) compared to the corresponding adenine nucleotide (right).

The replacement of a homo-DNA adenine nucleotide against the corresponding diaminopyrimidine nucleotide in a central position had a much stronger influence on the homo-DNA duplex stability than the replacement of a homo-DNA adenine nucleotide near the 4'-end of the homo-DNA-oligonucleotide: In the temperature dependent UV-melting curve of the octamer ddGlc[UAUD$(UA)_2$] an increasing hyperchromicity value for increasing oligonucleotide concentration was detected: The 14.9 µM solution showed a hyperchromicity of 2 % (**Fig. 41**, blue melting curve), this value was increased to more than 3 % for the 22.7 µM and the 34.4 µM oligonucleotide solution (**Fig. 41**, black melting curve, green melting curve). This observation together with the low hyperchromicity values observed for ddGlc[UAUD$(UA)_2$] compared to ddGlc[UD$(UA)_3$] indicated that even with the 34.4 µM solution the homo-DNA-oligonucleotide was partially melted at $t$ = 2.5 °C, the lowest measured temperature (water based temperature control unit). For the octamer ddGlc[UAUD$(UA)_2$] no calculation of *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) was performed, because the prerequisite for this determination is the observation of the completely unmelted duplex (method see[83b,84]) .

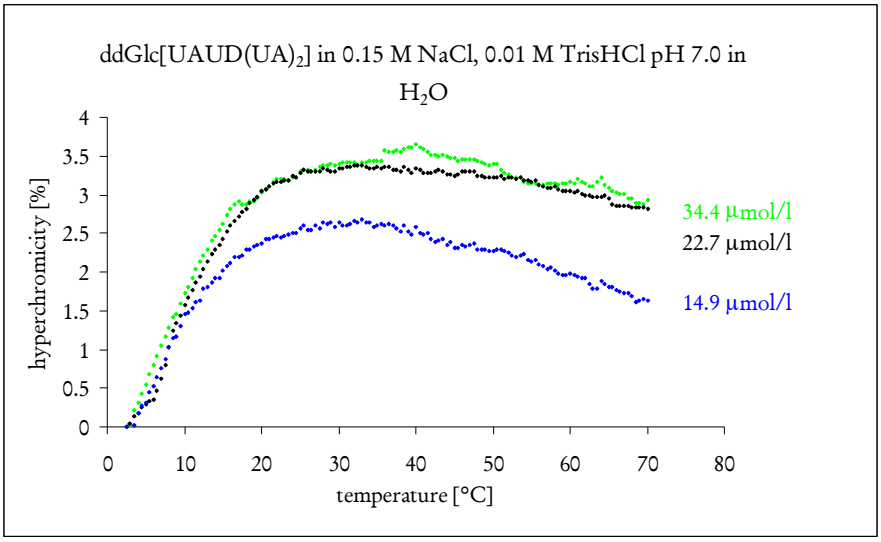



**Fig. 41** UV-melting curve of the octanucleotide ddGlc[UAUD(UA)$_2$] in 0.15 M NaCl 0.01 M TrisHCl, pH 7.0. The increasing hyperchromicity values with increasing oligonucleotidconcentration indicate that the octamer was already partially melted at the lowest measured temperature ($t$ = 2.5 °C).

A comparison of the melting curves of the unmodified ddGlc[(UA)$_4$] with ddGlc[UD(UA)$_3$] and ddGlc[UAUD(UA)$_2$] showed that the duplex destabilization of a 2,4-diaminopyrimidine nucleotide is much stronger in a central position than near the 4'-end of the homo-DNA-oligonucleotide (**Fig. 42**).

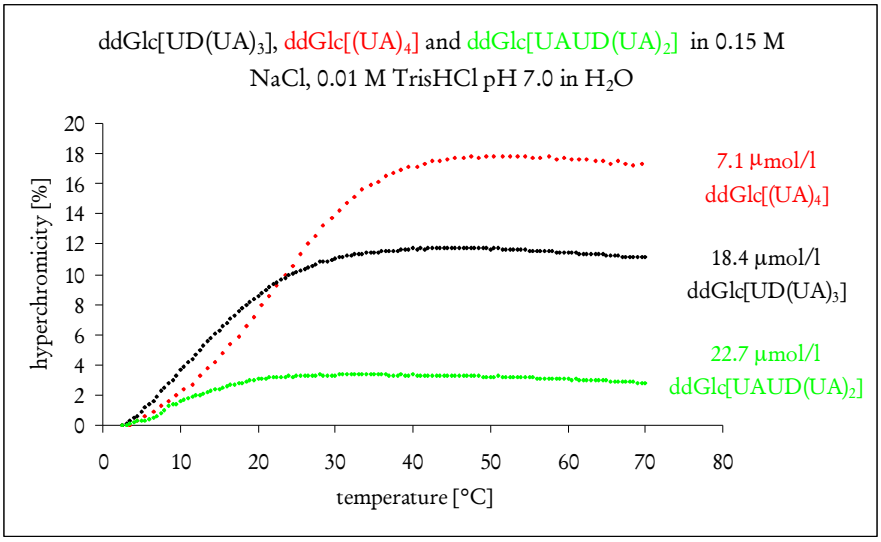
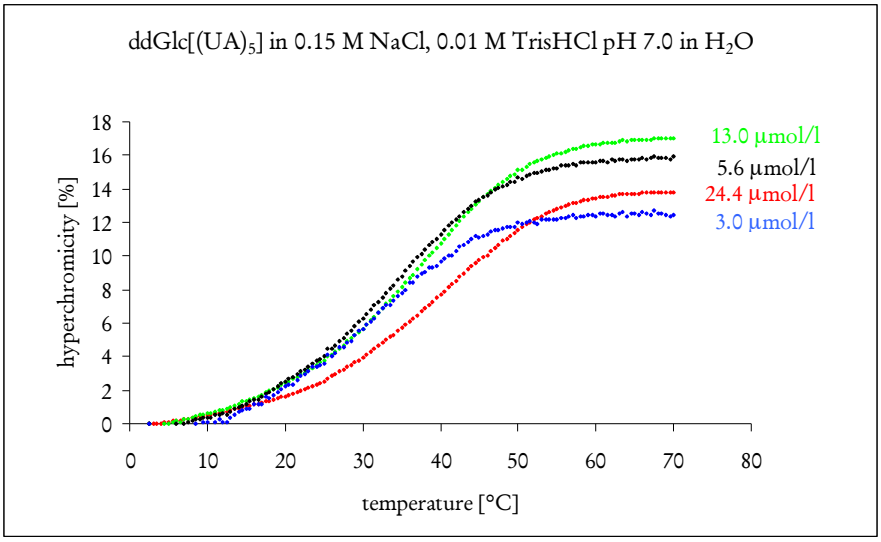


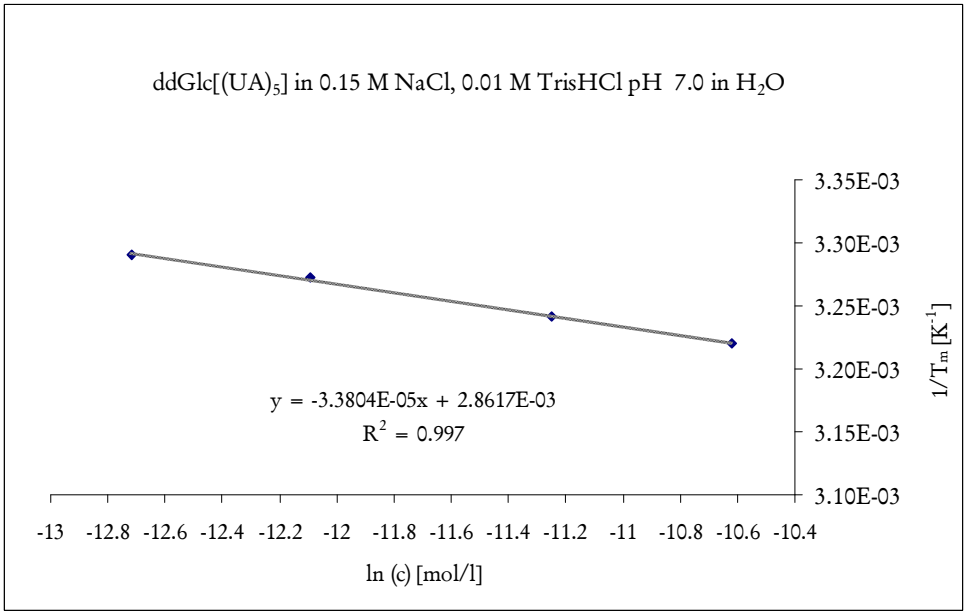

**Fig. 42** UV-melting curve of the octanucleotides ddGlc[$(UA)_4$], ddGlc[$UD(UA)_3$] and ddGlc[$UAUD(UA)_2$] in 0.15 M NaCl 0.01 M TrisHCl, pH 7.0.

The dramatic decrease of duplex stability by the incorporation of a 2,4-diaminopyrimidine nucleotide in a central position of the self-complementary homo-DNA octanucleotide ddGlc[$(UA)_4$] let us investigate the destabilizing effect of central 2,4-diaminopyrimidine nucleotides in longer homo-DNA oligonucleotides. If the 2,4-diaminopyrimidine nucleotide was incorporated near the 4'-end of the homo-DNA oligonucleotide the duplex destabilization by the 2,4-diaminopyrimidine nucleotide is only due to entropic but not due to enthalpic reasons.

The decamer ddGlc[$(UA)_5$] was analyzed according to similar methods described for other homo-DNA oligonucleotides (see ddGlc[$(UA)_4$]). A sigmoid melting curve was obtained for ddGlc[$(UA)_5$] with hyperchromicity values between 13 % and 17 % for oligonucleotide concentrations between 24.4 µM and 3.0 µM (**Fig. 43**, middle). $T_m$ values increased with increasing oligonucleotide concentration in solution. The $R^2$-value of 0.997 of the linear regression $1/T_m$ vs. ln(c) indicated a good fitting of the data and the determination of the *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$), resulted in $\Delta H$ = -246 kJ/mol and $\Delta S$ = -704 J/(mol*K) (**Fig. 43**).



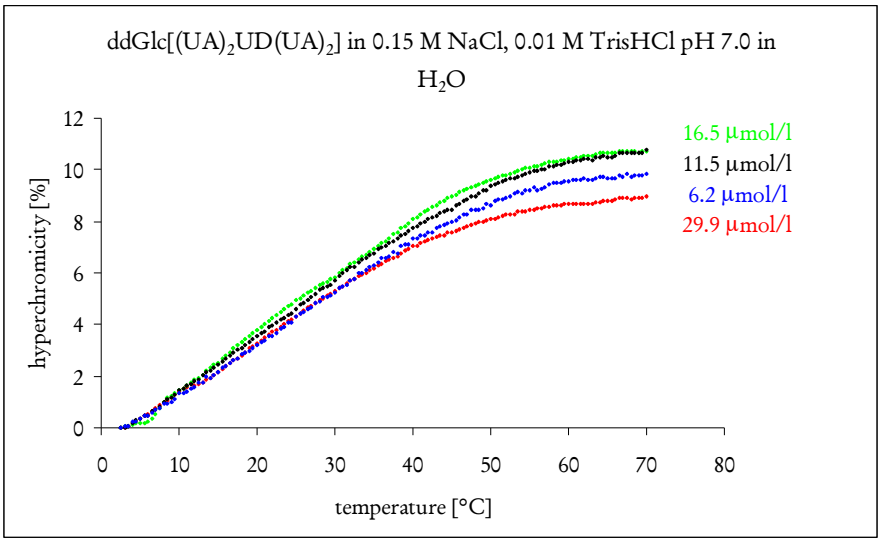

| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | Hyperchromicity (wavelength) [%] |
|---|---|---|---|
| ddGlc[$(UA)_5$] | 3.0 | 30.8 | 13 (260 nm) |
| ddGlc[$(UA)_5$] | 5.6 | 32.4 | 16 (260 nm) |
| ddGlc[$(UA)_5$] | 13.0 | 35.3 | 17 (260 nm) |
| ddGlc[$(UA)_5$] | 24.4 | 37.4 | 14 (260 nm) |




| Oligonucleotide | $\Delta H$ [kJ/mol] | $\Delta S$ [J/(mol*K)] | $\Delta G$ (T=25 °C) [kJ/mol] |
|---|---|---|---|
| ddGlc[$(UA)_5$] | -246 | -704 | -36.1 |

**Fig. 43** UV-melting curves of different oligonucleotide concentrations of the decanucleotide ddGlc[$(UA)_5$] in 0.15 M NaCl 0.01 M TrisHCl, pH 7.0 (top), determined $T_m$ values (values obtained from UV- melting) hyperchromicities (middle), and linear regression $1/T_m$ vs. ln(c) resulting in *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) (bottom).

From the modified decamer ddGlc[$(UA)_2UD(UA)_2$] a slightly sigmoid melting curve was obtained with constant hyperchromicity values of 10 % for oligonucleotide concentrations between 30.0 µM and 6.2 µM. The decamer ddGlc[$(UA)_2UD(UA)_2$] showed a decrease of $T_m$ with increasing oligonucleotide concentration in solution, clearly excluding an intermolecular homo-DNA duplex formation (**Fig. 44**).




| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | Hyperchromicity (wavelength) [%] |
|---|---|---|---|
| ddGlc[$(UA)_2UD(UA)_2$] | 6.2 | 28.1 | 10 (260 nm) |
| ddGlc[$(UA)_2UD(UA)_2$] | 11.5 | 27.5 | 11 (260 nm) |
| ddGlc[$(UA)_2UD(UA)_2$] | 16.5 | 25.8 | 11 (260 nm) |
| ddGlc[$(UA)_2UD(UA)_2$] | 29.9 | 24.2 | 9 (260 nm) |

**Fig. 44** UV-melting curves of different oligonucleotide concentrations of the decanucleotide ddGlc[$(UA)_2UD(UA)_2$] in 0.15 M NaCl 0.01 M TrisHCl, pH 7.0.

The hyperchromicity values of 10 % obtained for ddGlc[$(UA)_2UD(UA)_2$] were similar compared to hyperchromicity values obtained from ddGlc[$UD(UA)_3$]. The incorporation of a 2,4-diaminonucleotide in the middle of the oligonucleotide resulted in a much broader melting region between 2.5 °C and 55 °C, compared to the sharp melting of ddGlc[$(UA)_5$] (melting region between 20 °C and 50 °C, **Fig. 45**).

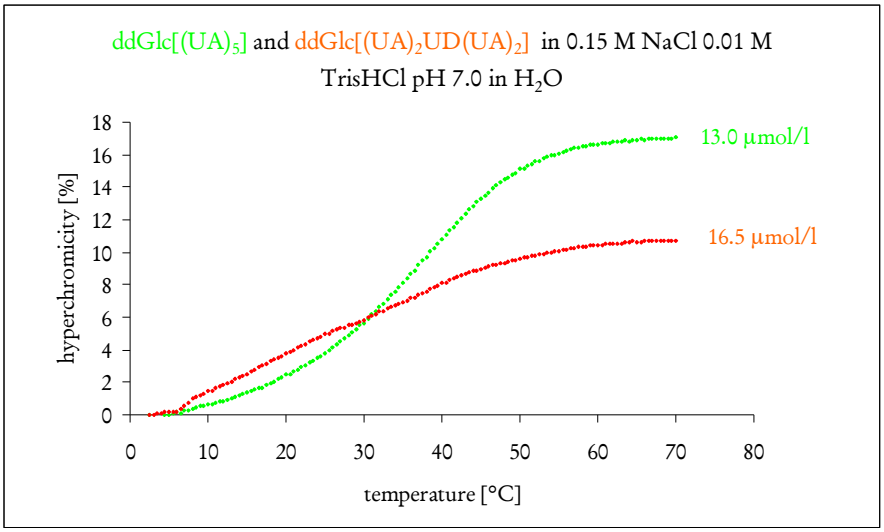
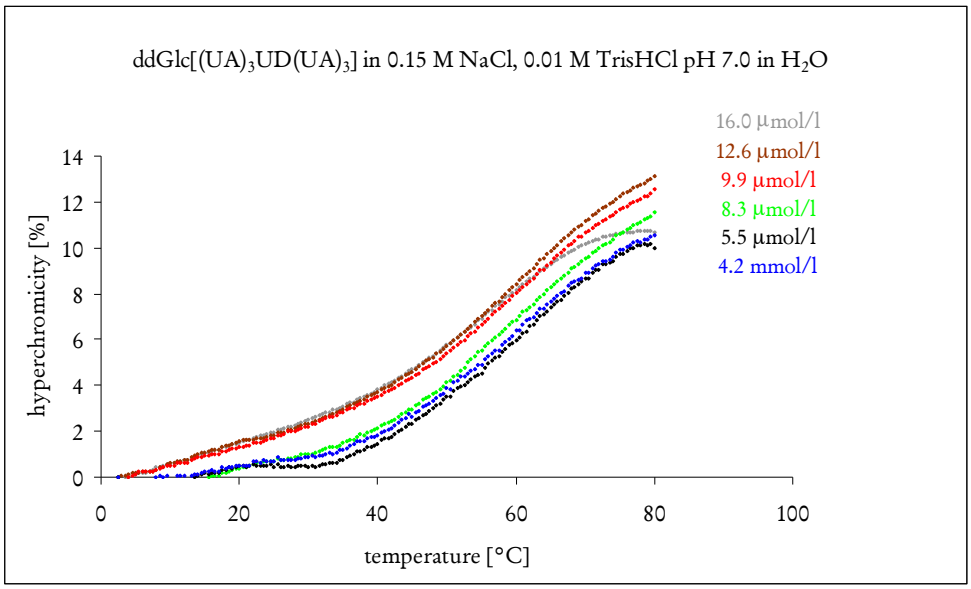



**Fig. 45** UV-melting curves of the decamers ddGlc[$(UA)_5$] and ddGlc[$(UA)_2UD(UA)_2$] in 0.15 M NaCl 0.01 M TrisHCl pH 7.0.

The interesting behavior of decreasing $T_m$ value with increasing oligonucleotide concentration in solution, observed in the decamer ddGlc[$(UA)_2UD(UA)_2$] led us to study the 14-mer ddGlc[$(UA)_3UD(UA)_3$]. For this homo-DNA oligonucleotide, a sigmoid melting curve was obtained with hyperchromicity values between 10 % and 13 % for an oligonucleotide concentration between 16.0 µM and 4.0 µM (**Fig. 46**, middle). Similar hyperchromicity values were already obtained for the decamer ddGlc[$(UA)_2UD(UA)_2$]. The interesting behavior of decreasing $T_m$ value with increasing oligonucleotide concentration, already observed in the decamer ddGlc[$(UA)_2UD(UA)_2$], was also observed in the 14-mer ddGlc[$(UA)_3UD(UA)_3$] for concentrations >6 µM. The decrease of the $T_m$ values with increasing oligonucleotide concentration was more pronounced with the 14-mer ddGlc[$(UA)_3UD(UA)_3$] compared to the

decamer ddGlc[$(UA)_2UD(UA)_2$] (ddGlc[$(UA)_3UD(UA)_3$], c = 8.3 µM, $T_m$ = 54.7 °C; c= 16 µM, $T_m$ = 48 °C; ddGlc[$(UA)_2UD(UA)_2$] c = 6.2 µM, $T_m$ = 28.1 °C, c = 16.5 µM, $T_m$ = 25.8 °C). However in the case of the 14-mer ddGlc[$(UA)_3UD(UA)_3$] for concentrations smaller than 6 µM no further concentration dependency of the $T_m$ values was observed. This experimental evidence indicated an intramolecular homo-DNA duplex formation in the 14-mer ddGlc[$(UA)_3UD(UA)_3$] with concentrations smaller than 6 µM.




| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | Hyperchromicity (wavelength) [%] |
|---|---|---|---|
| ddGlc[$(UA)_3UD(UA)_3$] | 4.2 | 57.4 | 11 (260 nm) |
| ddGlc[$(UA)_3UD(UA)_3$] | 5.5 | 57.3 | 10 (260 nm) |
| ddGlc[$(UA)_3UD(UA)_3$] | 8.3 | 54.7 | 12 (260 nm) |
| ddGlc[$(UA)_3UD(UA)_3$] | 9.9 | 53.7 | 13 (260 nm) |
| ddGlc[$(UA)_3UD(UA)_3$] | 12.6 | 52.9 | 13 (260 nm) |
| ddGlc[$(UA)_3UD(UA)_3$] | 16.0 | 48.0 | 11 (260 nm) |

**Fig. 46** UV-melting curves of different oligonucleotide concentrations of the oligonucleotide ddGlc[$(UA)_3$ $UD(UA)_3$] in 0.15 M NaCl 0.01 M TrisHCl, pH 7.0.

This data could be explained by an exclusive intramolecular interaction for oligonucleotide concentrations smaller than 6 µM. With increasing oligonucleotide concentration, a competitive intermolecular duplex formation occurs (**Fig. 47**).

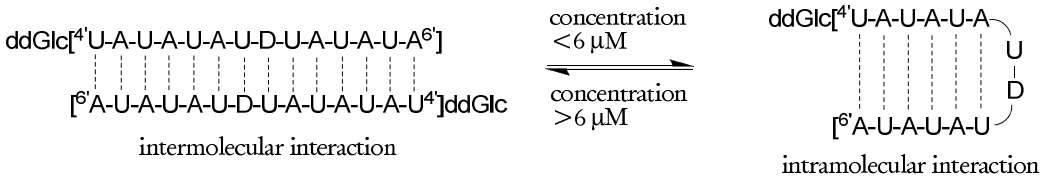



**Fig. 47** Inter- and intramolecular interaction of the 14-mer ddGlc[$(UA)_3UD(UA)_3$].

### 4.4.1.2 Homo-DNA sequences with the general formula ddGlc[$A_n$]

As mentioned in chapter 3.2.1 homo-DNA-[$A_n$]-oligonucleotides with n > 5 undergo a reverse-*Hoogsten* base pairing. If an adenine nucleotide is replaced by a 2,4-diaminopyrimidine nucleotide a reverse-*Hoogsten* base pairing is still possible. A replacement of both adenine nucleotides by 2,4-diaminopyrimidine nucleotides still allows a 2,4-diaminopyrimidine-2,4-diaminopyrimidine base pairing (**Fig. 48**).

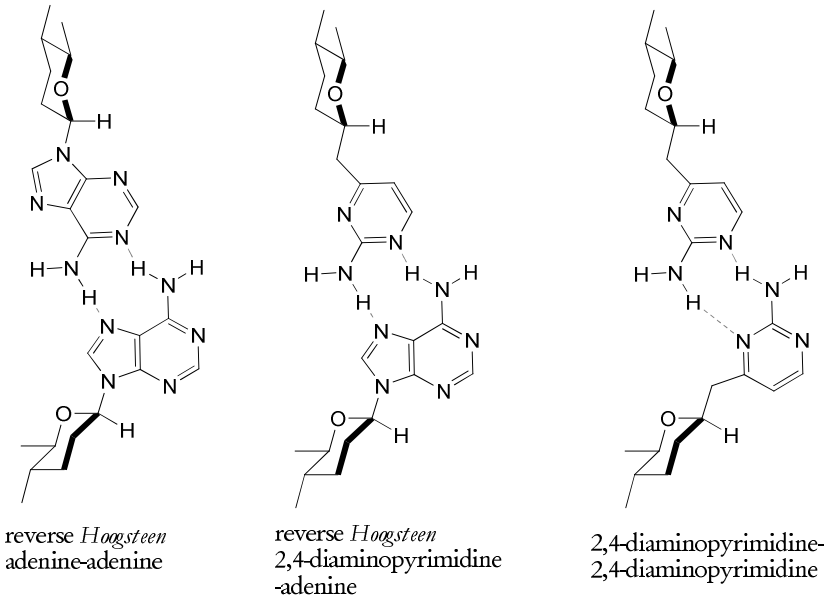
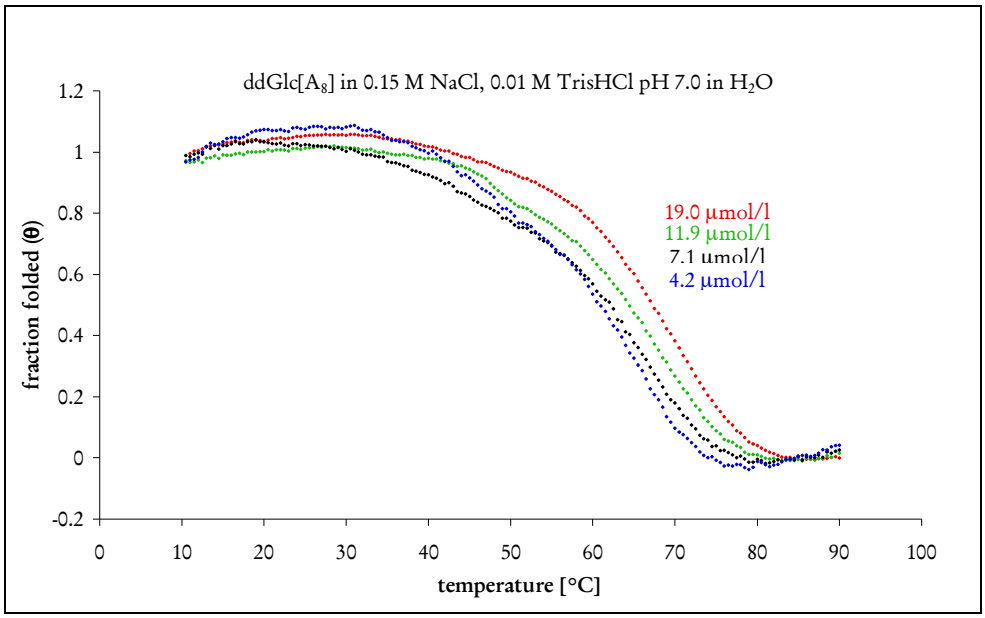
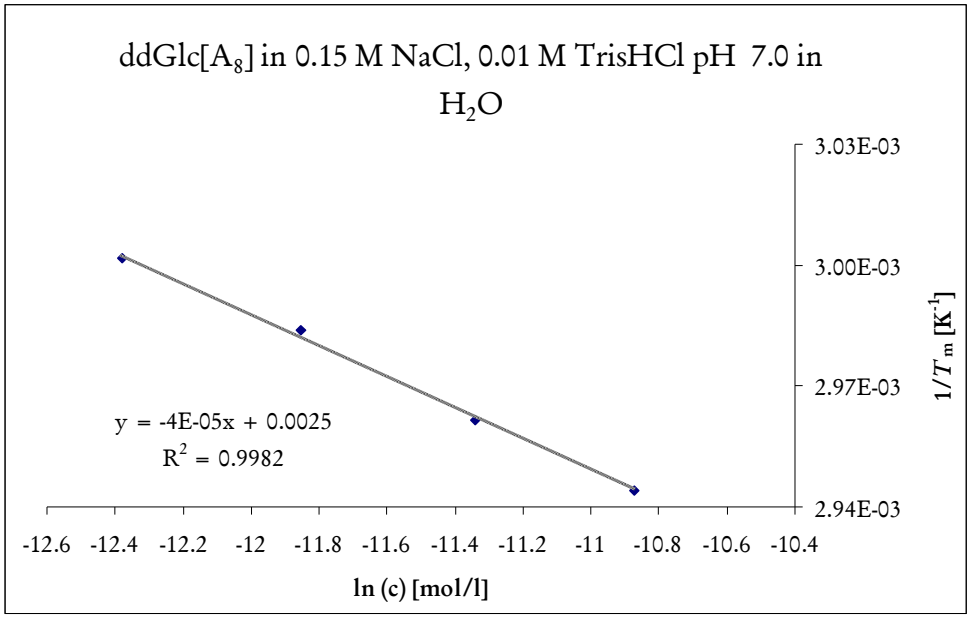



**Fig. 48** Reverse *Hoogsteen* adenine-adenine, reverse *Hoogsteen* 2,4-diaminopyrimidine-adenine and 2,4-diaminopyrimidine-2,4-diaminopyrimidine analogue of the reverse *Hoogsteen* base pairings.

For comparison data, the octanucleotide ddGlc[$A_8$] was investigated. As expected for an intermolecular duplex formation the $T_m$ values increased with increasing oligonucleotide concentration in solution. The $R^2$-value of 0.998 of the linear regression $1/T_m$ vs. ln(c) indicated a good fitting of the data and the determination of the *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$), resulted in $\Delta H$ = -208 kJ/mol and $\Delta S$ = -520 J/(mol*K) (**Fig. 49**).




| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | Hyperchromicity (wavelength) [%] |
|---|---|---|---|
| ddGlc[$A_8$] | 19.0 | 66.5 | 7 (260 nm) |
| ddGlc[$A_8$] | 11.9 | 64.5 | 7 (260 nm) |
| ddGlc[$A_8$] | 7.1 | 62.0 | 7 (260 nm) |
| ddGlc[$A_8$] | 4.2 | 60.0 | 7 (260 nm) |




| **Oligonucleotide** | ***ΔH* [kJ/mol]** | ***ΔS* [J/(mol*K)]** | ***ΔG* (T=25 °C) [kJ/mol]** |
|---|---|---|---|
| ddGlc[$A_8$] | -208 | -520 | -52.9 |

**Fig. 49** UV-melting curve (at 260 nm) of different oligonucleotide concentrations of the oligonucleotide ddGlc[$A_8$] in 0.15 M NaCl 0.01 M TrisHCl, pH 7.0 and determination of the *Van't Hoff*-pairing enthalpies and – entropies.

By a replacement of all adenine nucleotides by 2,4-diaminopyrimidine nucleotides the following effects were observed. The melting curve of the nonanucleotide ddGlc[$UD_8$] showed a much weaker interaction compared to the octanucleotide ddGlc[$A_8$] (**Fig. 50**). The low signal to noise ratio was due to the low extinction coefficient of the the nonanucleotide ddGlc[$UD_8$] (ε (290 nm) 2300).

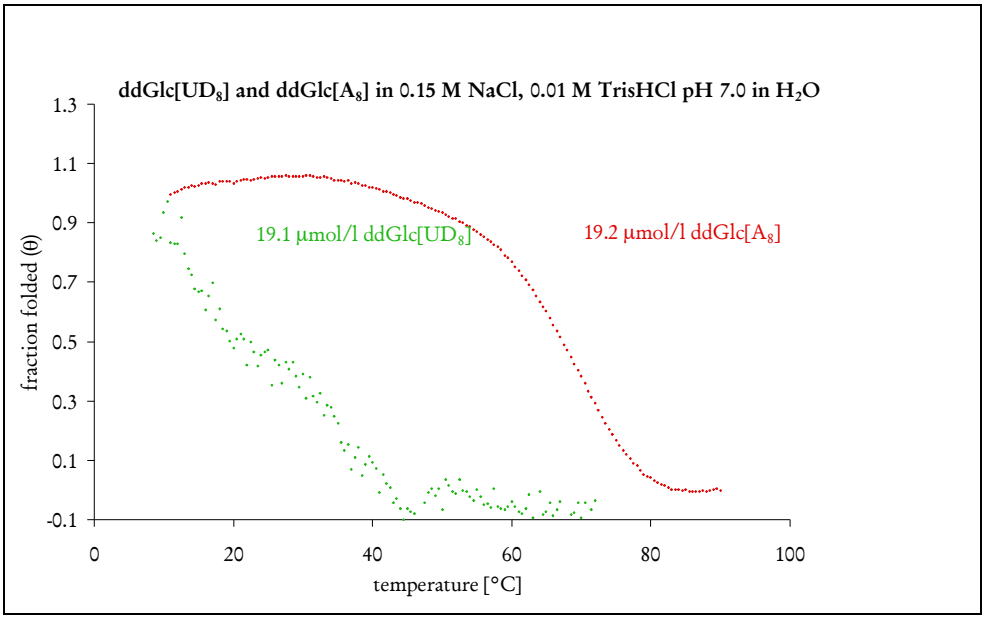



**Fig. 50** UV-melting curve (at 290 nm) of the nonanucleotide ddGlc[$UD_8$] in 0.15 M NaCl 0.01 M TrisHCl, pH 7.0 compared to the UV-melting curve (at 260 nm) of the octanucleotide ddGlc[$A_8$] in 0.15 M NaCl 0.01 M TrisHCl, pH 7.0.

To study the influence of a replacement of single homo-DNA adenine nucleotide against a 2,4-diaminopyrimidine nucleotide in the octamer ddGlc[$A_8$] a study of the octamer ddGlc[$A_2DA_5$] was performed. In this oligonucleotide, two reverse *Hoogsteen* adenine-adenine base pairings are replaced against two 2,4-diaminopyrimidine-adenine base pairings. (**Fig. 51**).

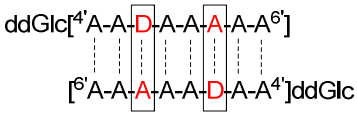
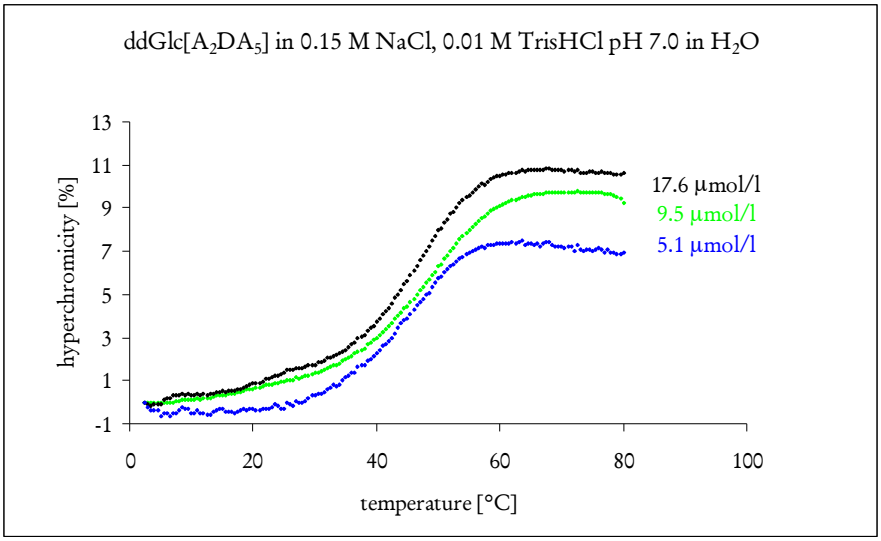


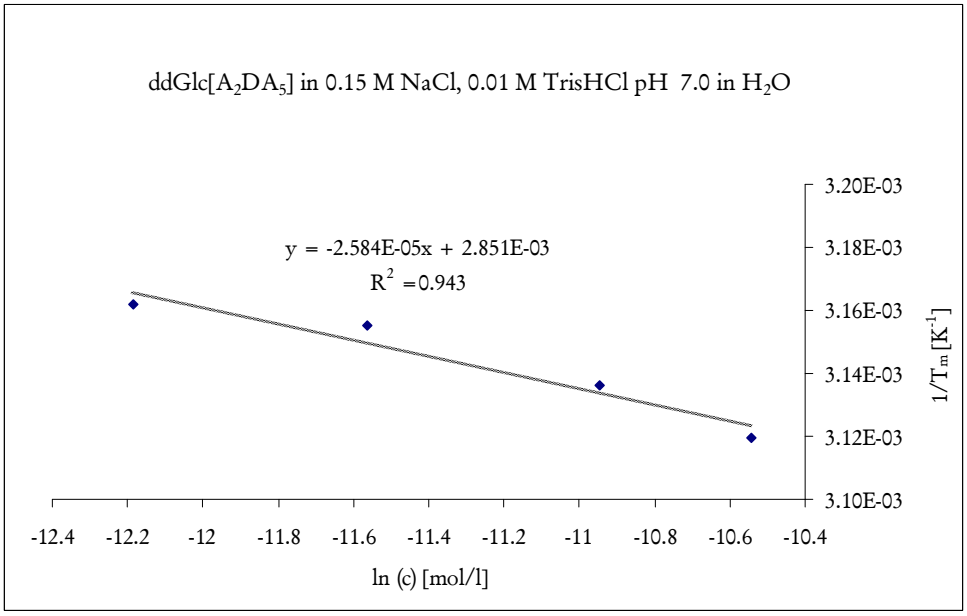

**Fig. 51** Base pairing in the octamer ddGlc[$A_2DA_5$]

The octamer ddGlc[$A_2DA_5$] showed a sigmoid melting curve with hyperchromicity values between 8 % and 11 % for oligonucleotide concentrations between 26.4 µM and 5.1 µM (**Fig. 52**, middle). As expected for intermolecular interactions the $T_m$ values increased with increasing oligonucleotide concentration in solution. The $R^2$-value of 0.943 of the linear regression $1/T_m$ vs. ln(c) indicated an acceptable fitting of the data and the determination of the *Van't Hoff*-pairing

enthalpies ($\Delta H$) and –entropies ($\Delta S$), resulted in $\Delta H$ = -322 kJ/mol and $\Delta S$ = -917 J/(mol*K) (**Fig. 52**).



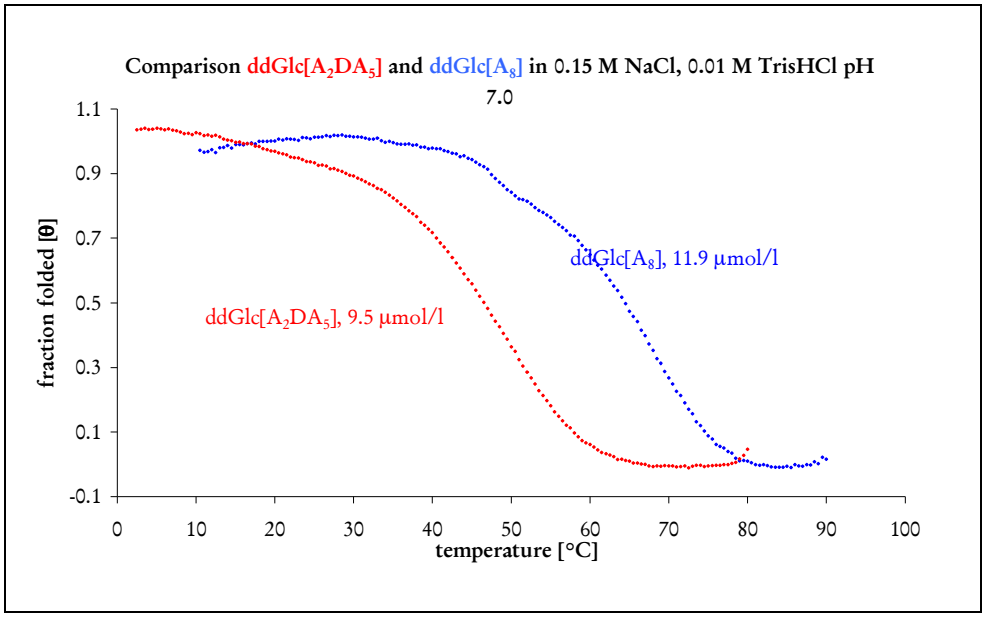

| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | Hyperchromicity (wavelength) [%] |
|---|---|---|---|
| ddGlc[$A_2DA_5$] | 26.4 | 47.7 | 9 (260 nm) |
| ddGlc[$A_2DA_5$] | 17.6 | 45.7 | 9 (260 nm) |
| ddGlc[$A_2DA_5$] | 9.5 | 43.8 | 11 (260 nm) |
| ddGlc[$A_2DA_5$] | 5.1 | 43.1 | 8 (260 nm) |



| Oligonucleotide | *ΔH* [kJ/mol] | *ΔS* [J/(mol*K)] | *ΔG* (T=25 °C) [kJ/mol] |
|---|---|---|---|
| ddGlc[$A_2DA_5$] | -322 | -917 | -48.3 |

**Fig. 52** UV-melting curves of different oligonucleotide concentrations of the octanucleotide ddGlc[$A_2DA_5$] in 0.15 M NaCl 0.01 M TrisHCl, pH 7.0 (top), determined $T_m$ values (values obtained from UV- melting) hyperchromicities (middle), and linear regression $1/T_m$ vs. ln(c) resulting in *Van't Hoff*-pairing enthalpies (*ΔH*) and –entropies (*ΔS*) (bottom).

A comparison of melting curves obtained for the octanucleotides ddGlc[$A_8$] and ddGlc[$A_2DA_5$] showed a decrease of $T_m$ from 64.5 °C for the octanucleotide ddGlc[$A_8$] (concentration 11.9 µM) to 43.8 °C for ddGlc[$A_2DA_5$] (concentration 9.5 µM). As already observed in the octamer ddGlc[$UD(UA)_3$] the destabilization of the octamer ddGlc[$A_2DA_5$] in comparison to the octamer ddGlc[$A_8$] is only due to entropic reasons but not to enthalpic reasons. In the case of ddGlc[$A_2DA_5$] even an enthalpic stabilization compared to ddGlc[$A_8$] was found (**Fig. 53**).




| Oligonucleotide | *ΔH* [kJ/mol] | *ΔS* [J/(mol*K)] | *ΔG* (T=25 °C) [kJ/mol] |
|---|---|---|---|
| ddGlc[$A_8$] | -208 | -520 | -52.9 |
| ddGlc[$A_2DA_5$] | -322 | -917 | -48.3 |

**Fig. 53** UV-melting curves of the octanucleotides ddGlc[$A_2DA_5$] and ddGlc[$A_8$] in concentrations of 9.5 and 11.9 µM respectively (values obtained in 0.15 M NaCl, TrisHCl pH 7.0) and *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) (bottom).

### 4.4.2 Non-self-complementary homo-DNA sequences with the general formula ddGlc[$^{4'}$CAUA-X-GUGA$^{6'}$], ddGlc[$^{4'}$UCAC-X-UAUG$^{6'}$]

For obtaining comparable data a study of the pairing behavior of a 1:1 mixture of the two unmodified homo-DNA oligonucleotides ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$] and ddGlc[$^{4'}$UCAC**U**UAUG$^{6'}$] was performed (**Fig. 54**).

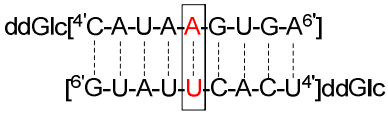
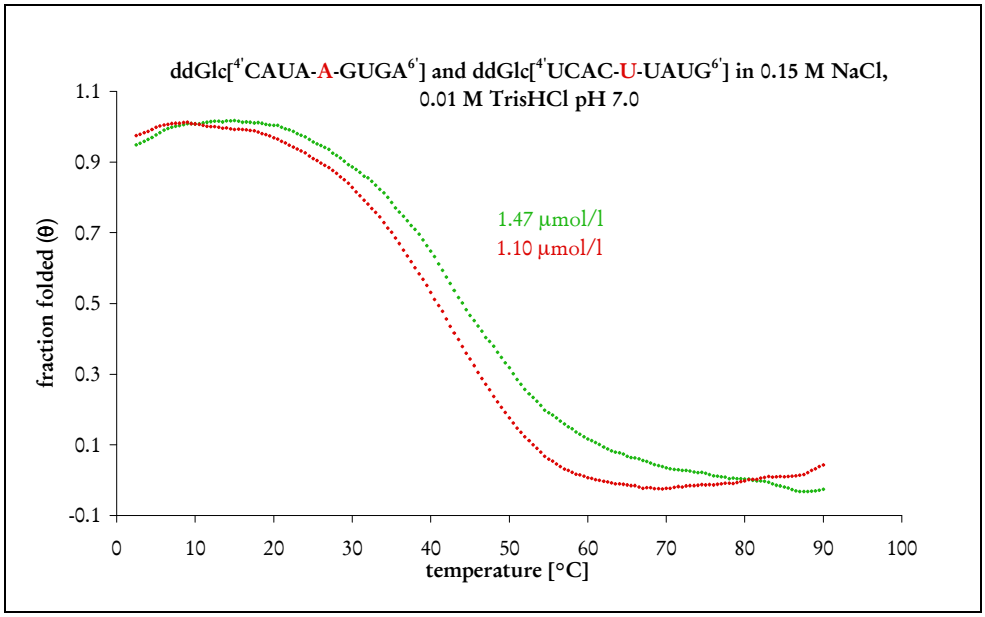


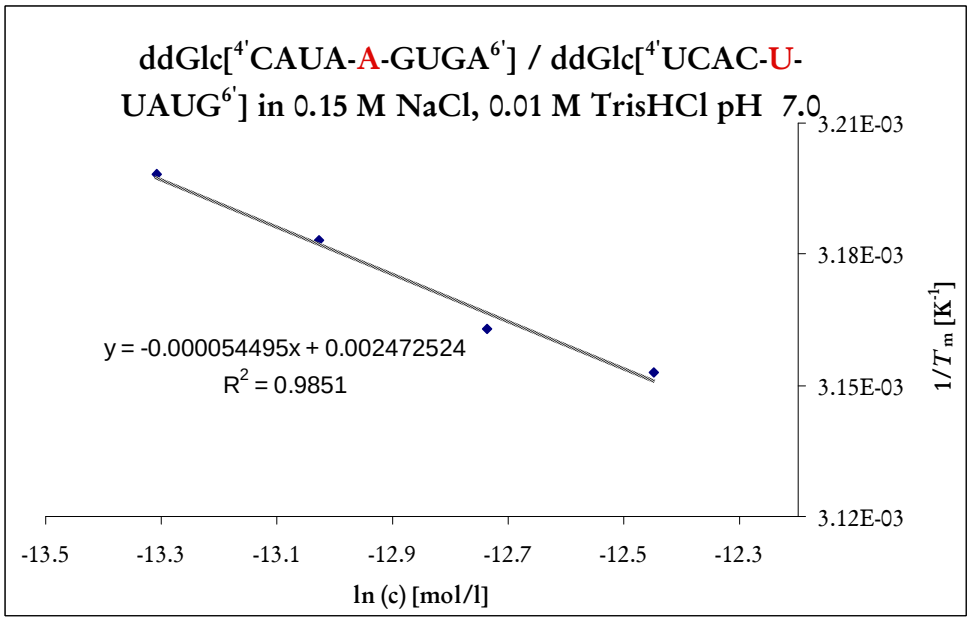

**Fig. 54** *Watson-Crick* base pairing in the non self-complementary sequences ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$] and ddGlc[$^{4'}$UCAC**U**UAUG$^{6'}$].

The nonamers ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$] and ddGlc[$^{4'}$UCAC**U**UAUG$^{6'}$] showed a sigmoid melting curve with hyperchromicity values between 8 % and 10 % for oligonucleotide concentrations between 1.96 µM and 0.83 µM.

The $T_m$ values were increased with increasing oligonucleotide concentration in solution (**Fig. 55**). The $R^2$-value of 0.985 of the linear regression $1/T_m$ vs. ln(c) indicated a good fitting of the data and the determination of the *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$), resulted in $\Delta H$ = -152.6 kJ/mol and $\Delta S$ = -366 J/(mol*K) (**Fig. 55**).



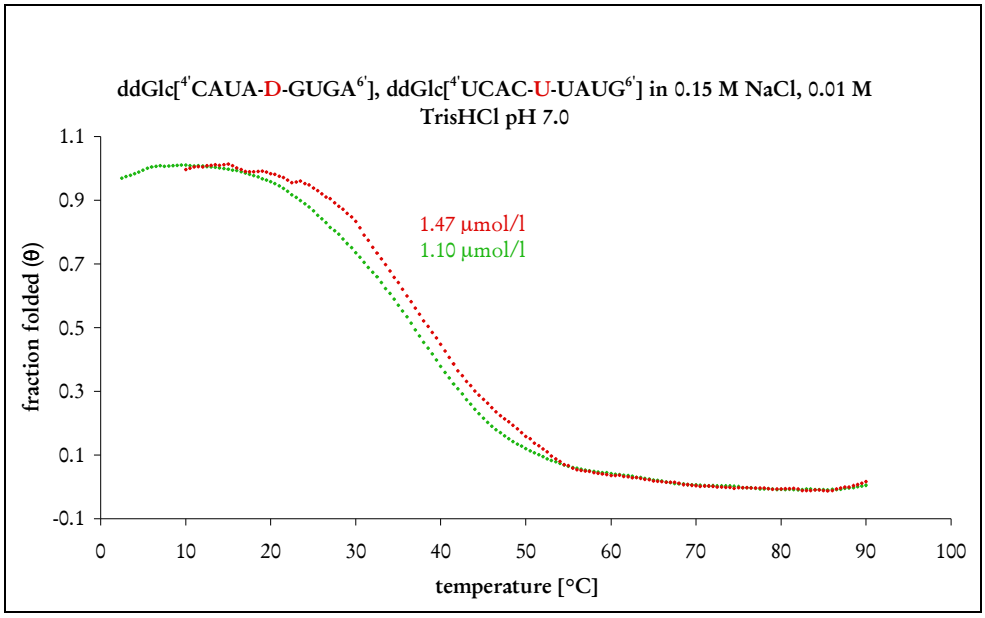

| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | Hyperchromicity (wavelength) [%] |
|---|---|---|---|
| ddGlc[4'CAUAAGUGA6']<br>ddGlc[4'UCACUUAUG6'] | 1.96 | 44.0 | 6 (260 nm) |
| ddGlc[4'CAUAAGUGA6']<br>ddGlc[4'UCACUUAUG6'] | 1.47 | 43.0 | 6 (260 nm) |
| ddGlc[4'CAUAAGUGA6']<br>ddGlc[4'UCACUUAUG6'] | 1.1 | 41.0 | 7 (260 nm) |
| ddGlc[4'CAUAAGUGA6']<br>ddGlc[4'UCACUUAUG6'] | 0.83 | 39.5 | 11 (260 nm) |



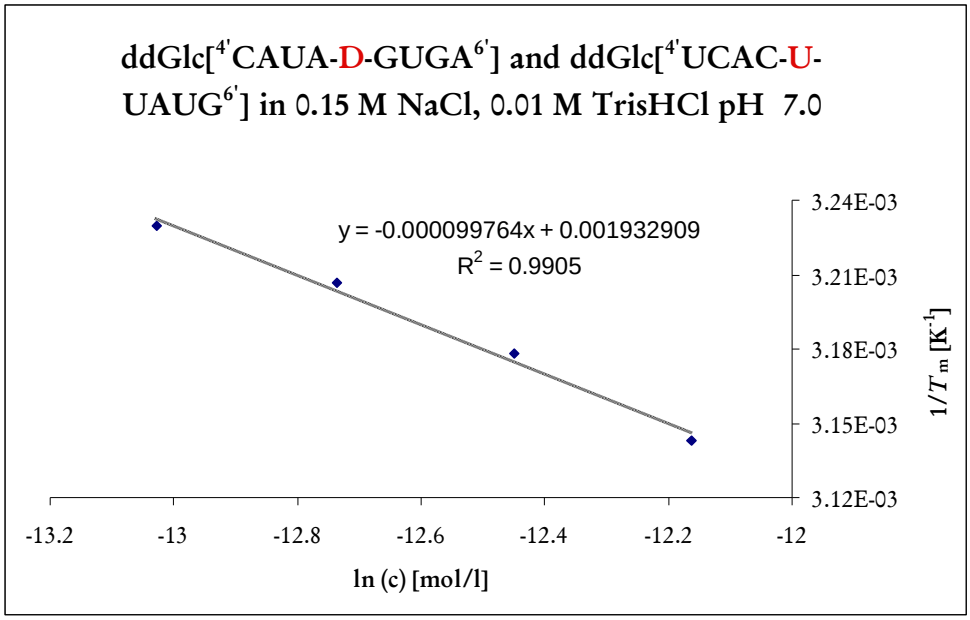
| Oligonucleotide | *ΔH* [kJ/mol] | *ΔS* [J/(mol*K)] | *ΔG* (T=25 °C) [kJ/mol] |
|---|---|---|---|
| ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**U**UAUG$^{6'}$] | -152.5 | -365.7 | -43.5 |

**Fig. 55** UV-melting curves of the nonanucleotides ddGlc[$^{4'}$CAUAAGUGA$^{6'}$] and ddGlc[$^{4'}$UCACUUAUG$^{6'}$] in concentrations of 1.47 µM and 0.83 µM (values obtained in 0.15 M NaCl, TrisHCl pH 7.0) (top), determined $T_m$ values (values obtained from UV- melting) and linear regression $1/T_m$ vs. ln(c) resulting in *Van't Hoff*-pairing enthalpies (*ΔH*) and –entropies (*ΔS*) (bottom).

The corresponding nonamers incorporating a 2,4-diaminopyrimidine-nucleoside ddGlc[$^{4'}$CAUA**D**GUGA$^{6'}$] and ddGlc[$^{4'}$UCAC**U**UAUG$^{6'}$] resulted in following melting curves (**Fig. 56**). A weak hyperchromicity of 2 to 5 % was found for oligonucleotide concentrations between 2.61 µM and 1.10 µM.



| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | Hyperchromicity (wavelength) [%] |
|---|---|---|---|
| ddGlc[$^{4'}$CAUA**D**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**U**UAUG$^{6'}$] | 2.61 | 45.0 | 2 (260 nm) |
| ddGlc[$^{4'}$CAUA**D**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**U**UAUG$^{6'}$] | 1.96 | 41.5 | 2 (260 nm) |
| ddGlc[$^{4'}$CAUA**D**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**U**UAUG$^{6'}$] | 1.47 | 38.7 | 3 (260 nm) |
| ddGlc[$^{4'}$CAUA**D**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**U**UAUG$^{6'}$] | 1.10 | 36.5 | 5 (260 nm) |




| Oligonucleotide | $\Delta H$ [kJ/mol] | $\Delta S$ [J/(mol*K)] | $\Delta G$ (T=25 °C) [kJ/mol] |
|---|---|---|---|
| ddGlc[$^{4'}$CAUA**D**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**U**UAUG$^{6'}$] | -83.3 | -149.6 | -38.7 |

**Fig. 56** UV-melting curves of the nonanucleotides ddGlc[$^{4'}$CAUADGUGA$^{6'}$] and ddGlc[$^{4'}$UCACUUAUG$^{6'}$] in concentrations of 1.47 µM and 1.10 µM (values obtained in 0.15 M NaCl in TrisHCl pH 7.0) (top) and linear regression $1/T_m$ vs. ln(c) resulting in *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) (bottom).

Evaluation of the data as described for the nonanucleotides ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$] and ddGlc[$^{4'}$UCAC**U**UAUG$^{6'}$] resulted in following thermodynamic parameters: *Van't Hoff*-pairing enthalpy $\Delta H$ = -83.3 kJ/mol and –entropy $\Delta S$ = -150 J/(mol*K). A comparison of melting curves

obtained for the nonamers ddGlc[CAUAAGUGA], ddGlc[UCACUUAUG] and ddGlc[CAUADGUGA], ddGlc[UCACUUAUG] showed a decrease of $T_m$ from 41.0 °C from the adenine-uracil homo-DNA base pairing to 36.5 °C for the 2,4-diaminopyrimidine-uracil base pairing. (concentrations 1.10 µM) (**Fig. 57**).

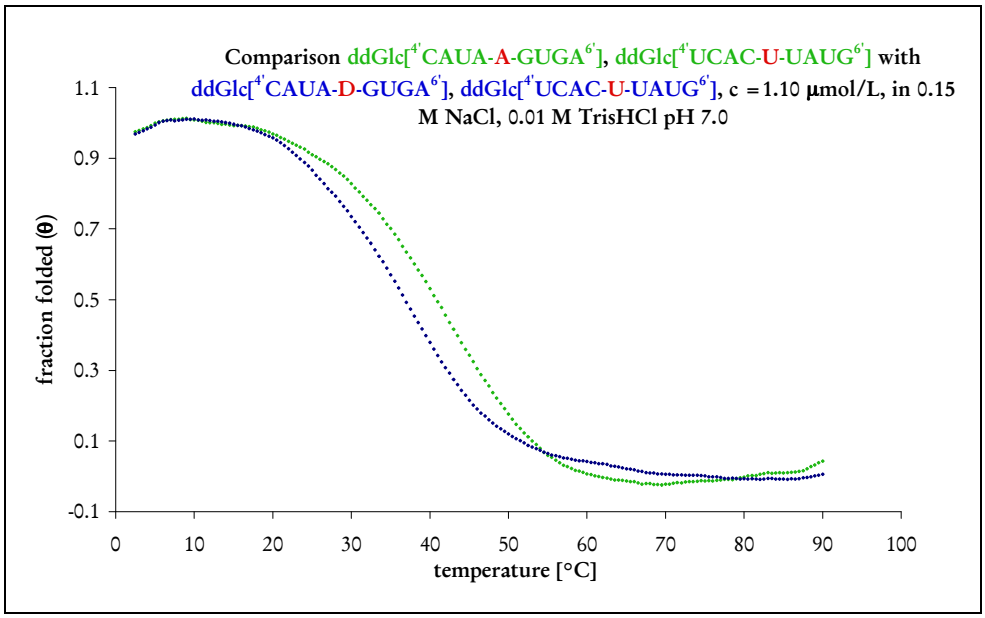



**Fig. 57** Comparison of UV-melting of ddGlc[$^{4'}$CAUAAGUGA$^{6'}$], ddGlc[$^{4'}$UCACUUAUG$^{6'}$] and ddGlc[$^{4'}$CAUADGUGA$^{6'}$], ddGlc[$^{4'}$UCACUUAUG$^{6'}$], c = 1.10 µM, in 0.15 M NaCl, TrisHCl pH 7.0.

The same evaluations were performed for the following interactions of homo-DNA-2,4-diaminopyrimidine nucleotide with the adenine, cytosine and guanine homo-DNA-nucleotide. Each base pairing A-A vs. D-A; A-C vs. D-C and A-G vs. D-G was evaluated. (**Fig. 58**).

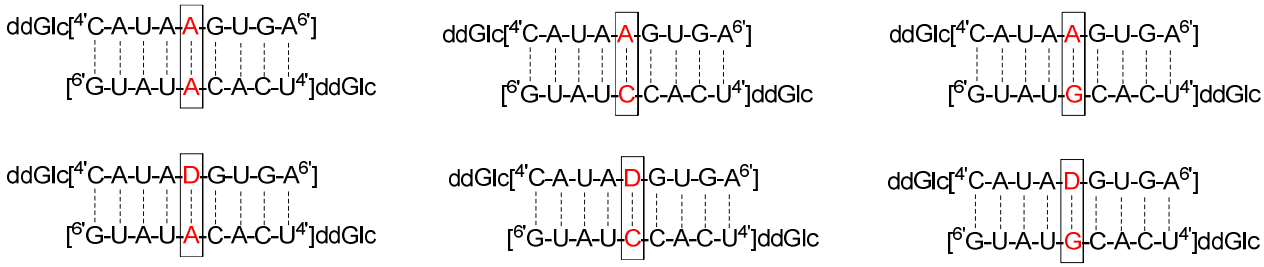
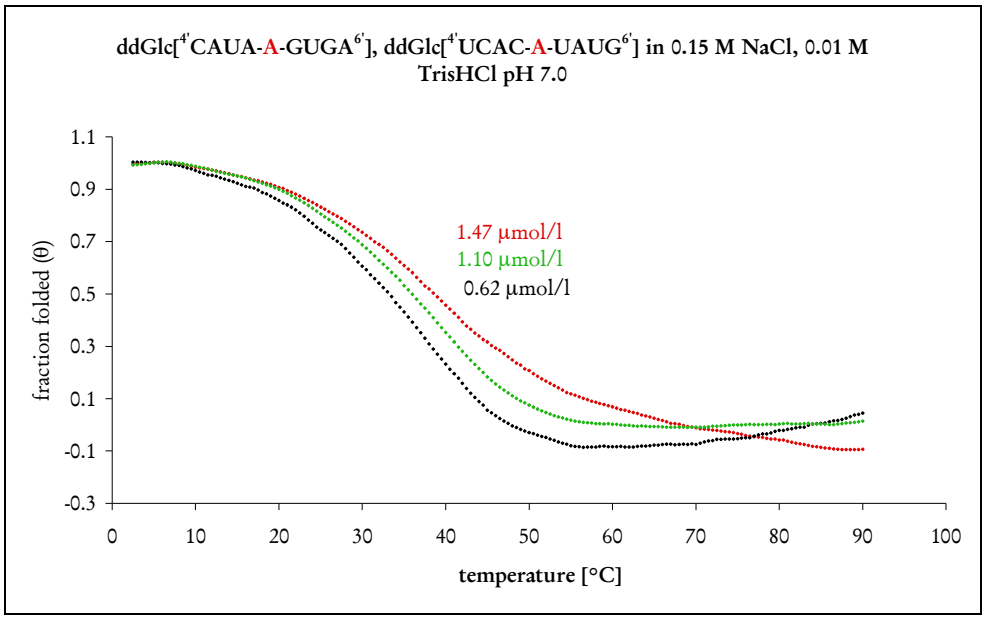
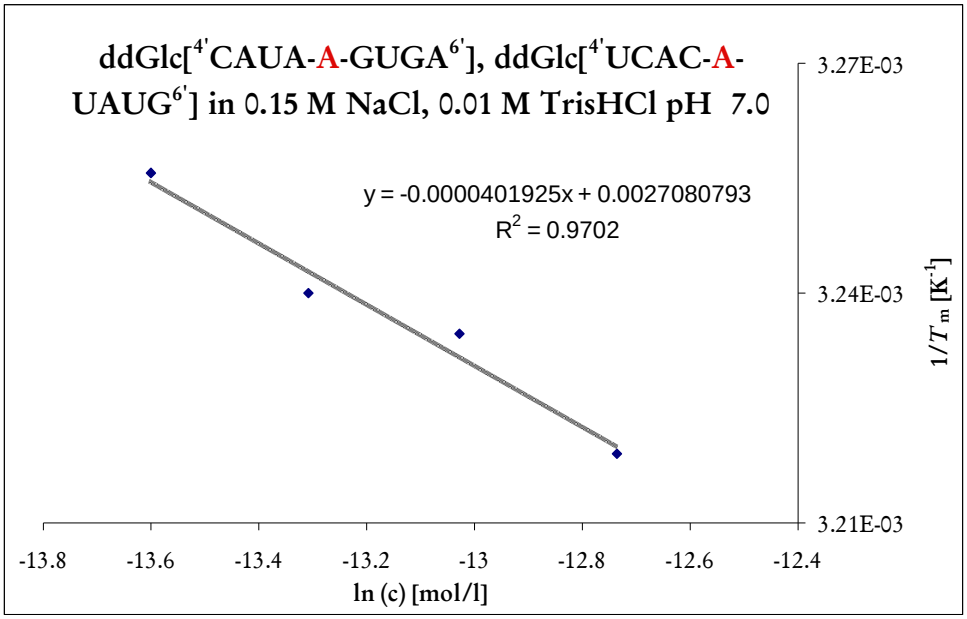



**Fig. 58** Homo-DNA-oligonucleotides investigated.

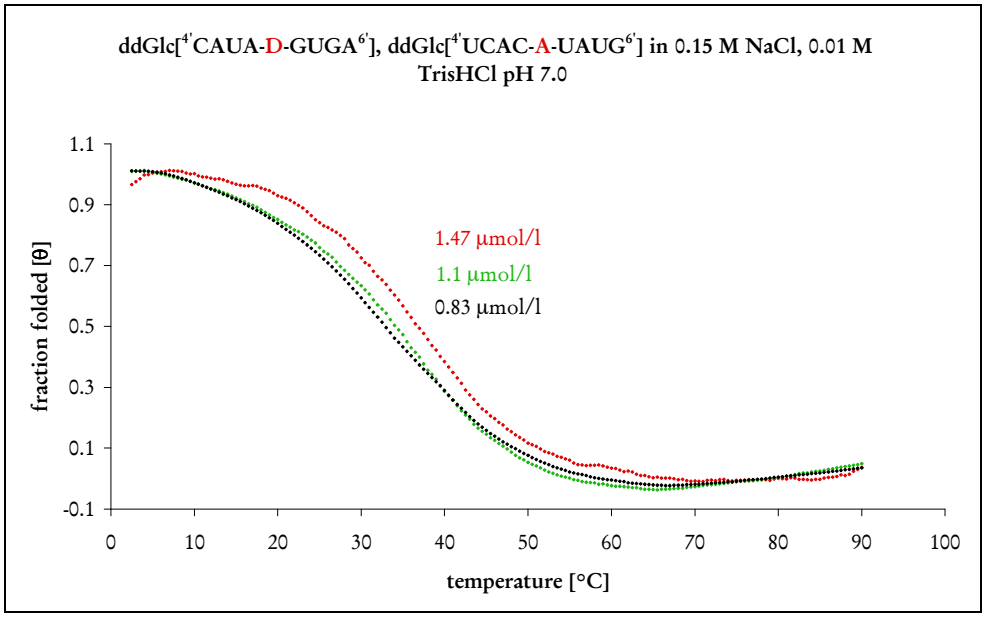
The resulting UV-melting, obtained $T_m$ values, linear regressions $1/T_m$ vs. ln (c) and *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) are shown in **Fig. 59 - Fig. 64**.

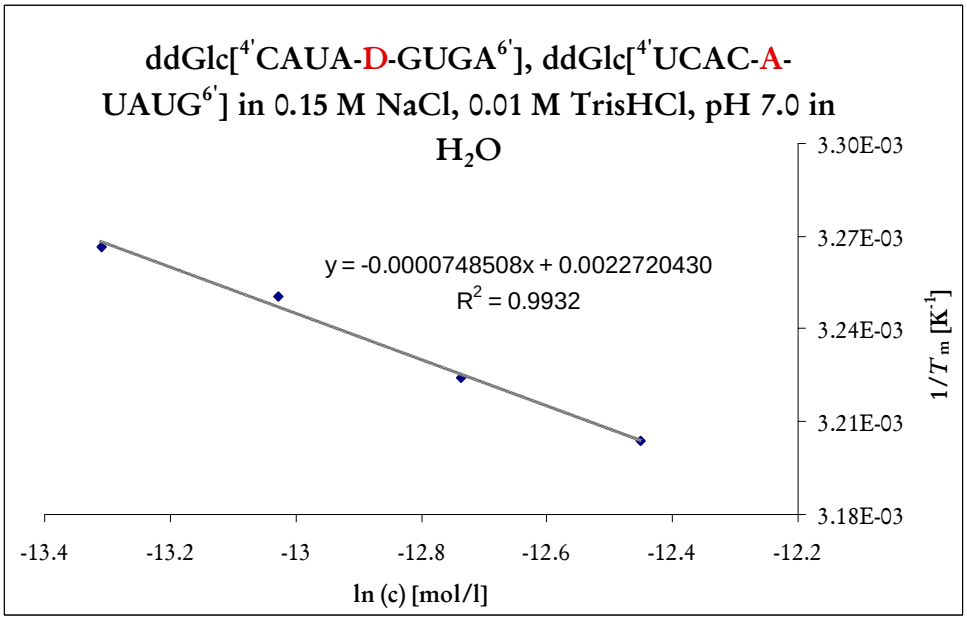





| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | $\Delta H$ [kJ/mol] | $\Delta S$ [J/(mol*K)] | $\Delta G$ (T=25 °C) [kJ/mol] |
|---|---|---|---|---|---|
| ddGlc[4'CAUAAGUGA6'] ddGlc[4'UCACAUAUG6'] | 1.47 | 37.5 | **-206.9** | **-548.7** | **-43.3** |
| ddGlc[4'CAUAAGUGA6'] ddGlc[4'UCACAUAUG6'] | 1.10 | 36 | | | |
| ddGlc[4'CAUAAGUGA6'] ddGlc[4'UCACAUAUG6'] | 0.83 | 35.5 | | | |
| ddGlc[4'CAUAAGUGA6'] ddGlc[4'UCACAUAUG6'] | 0.62 | 34.0 | | | |

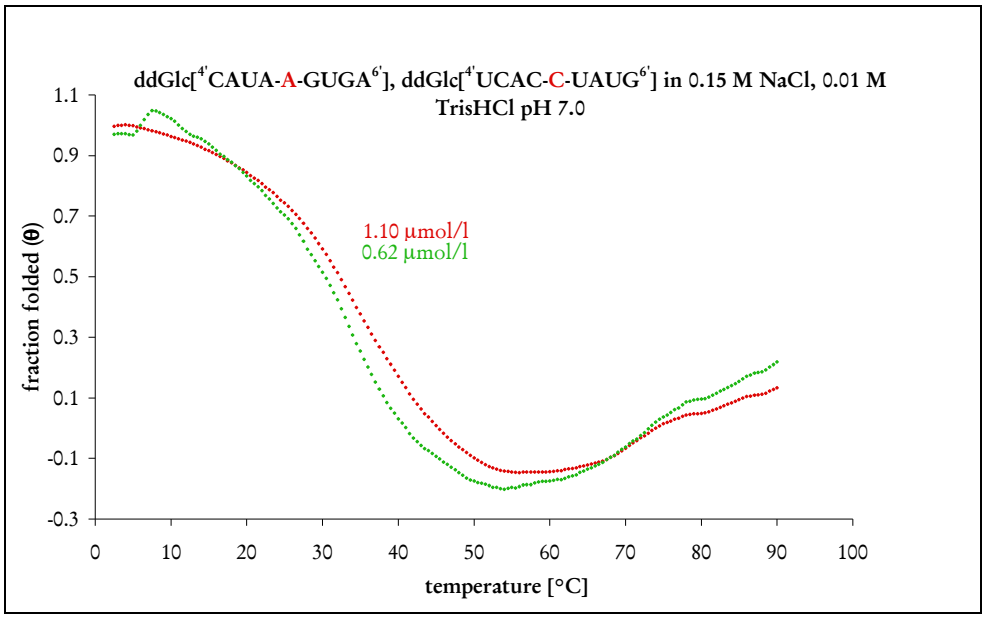
**Fig. 59** UV-melting curves (at 260 nm) of the nonanucleotides ddGlc[4'CAUAAGUGA6'] and ddGlc[4'UCACAUAUG6'] (values obtained in 0.15 M NaCl in TrisHCl pH 7.0) (top left) and linear regression $1/T_m$ vs. ln(c) resulting in *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) (bottom).

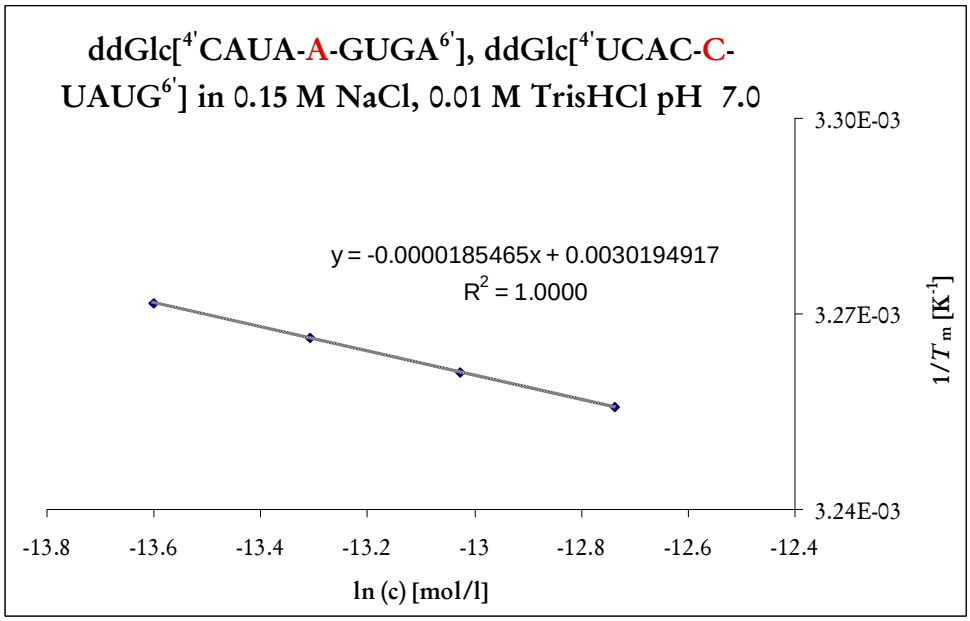



| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | $\Delta H$ [kJ/mol] | $\Delta S$ [J/(mol*K)] | $\Delta G$ (T=25 °C) [kJ/mol] |
|---|---|---|---|---|---|
| ddGlc[4'CAUADGUGA6']<br>ddGlc[4'UCACAUAUG6'] | 1.96 | 39.0 | **-111.1** | **-240.9** | **-39.3** |
| ddGlc[4'CAUADGUGA6']<br>ddGlc[4'UCACAUAUG6'] | 1.47 | 37.0 | | | |
| ddGlc[4'CAUADGUGA6']<br>ddGlc[4'UCACAUAUG6'] | 1.10 | 34.5 | | | |
| ddGlc[4'CAUADGUGA6']<br>ddGlc[4'UCACAUAUG6'] | 0.83 | 33.0 | | | |

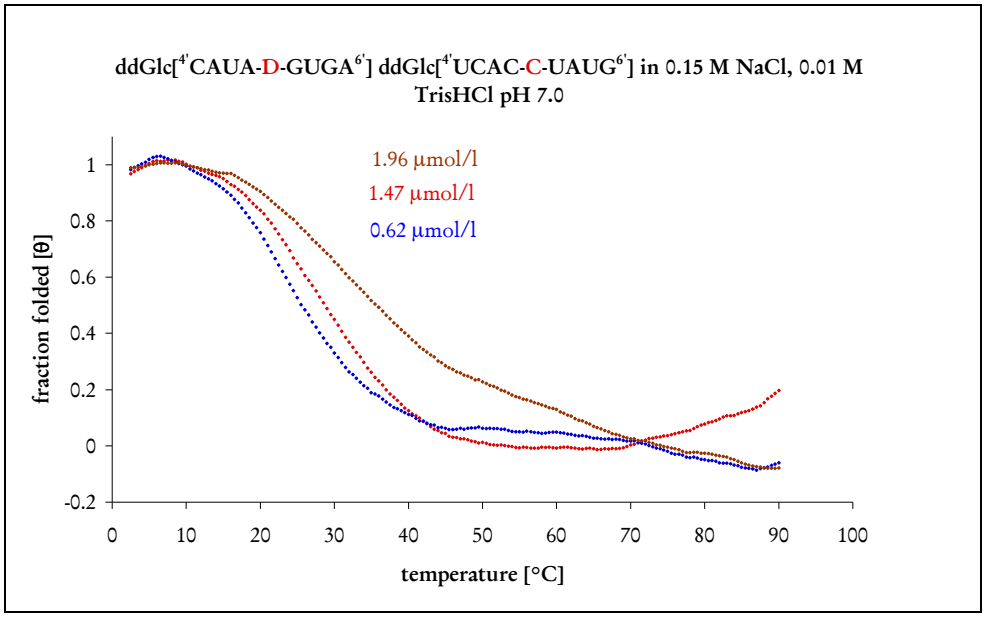
**Fig. 60** UV-melting curves (at 260 nm) of the nonanucleotides ddGlc[$^{4'}$CAUADGUGA$^{6'}$] and ddGlc[$^{4'}$UCACAUAUG$^{6'}$] (values obtained in 0.15 M NaCl, TrisHCl pH 7.0) (top left) and linear regression $1/T_m$ vs. ln(c) resulting in *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) (bottom).

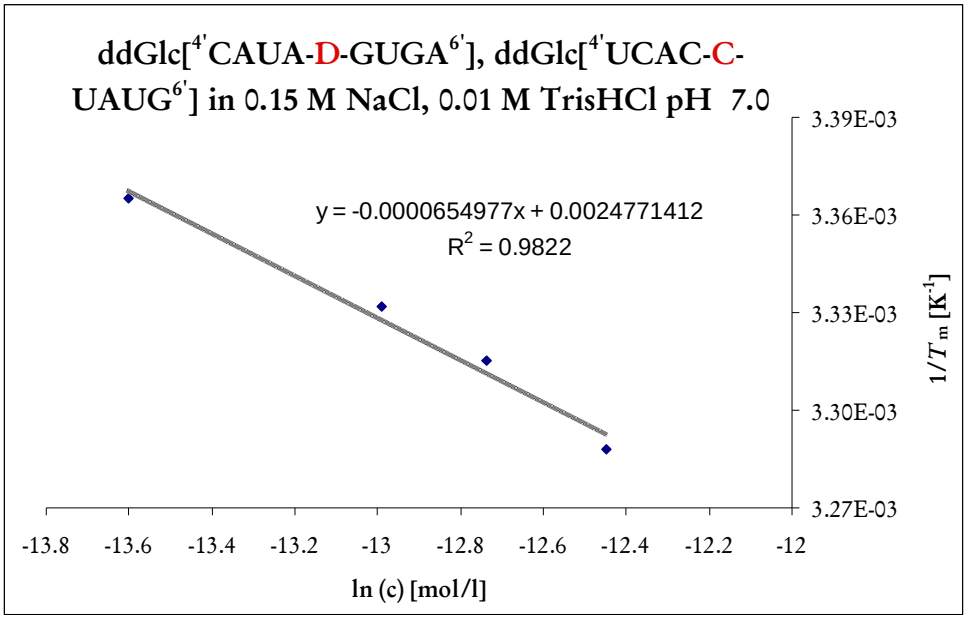


| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | $\Delta H$ [kJ/mol] | $\Delta S$ [J/(mol*K)] | $\Delta G$ (T=25 °C) [kJ/mol] |
|---|---|---|---|---|---|
| ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**C**UAUG$^{6'}$] | 1.47 | 34.0 | **-448.3** | **-1342.1** | **-48.1** |
| ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**C**UAUG$^{6'}$] | 1.10 | 33.5 | | | |
| ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**C**UAUG$^{6'}$] | 0.83 | 33.0 | | | |
| ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**C**UAUG$^{6'}$] | 0.62 | 32.5 | | | |

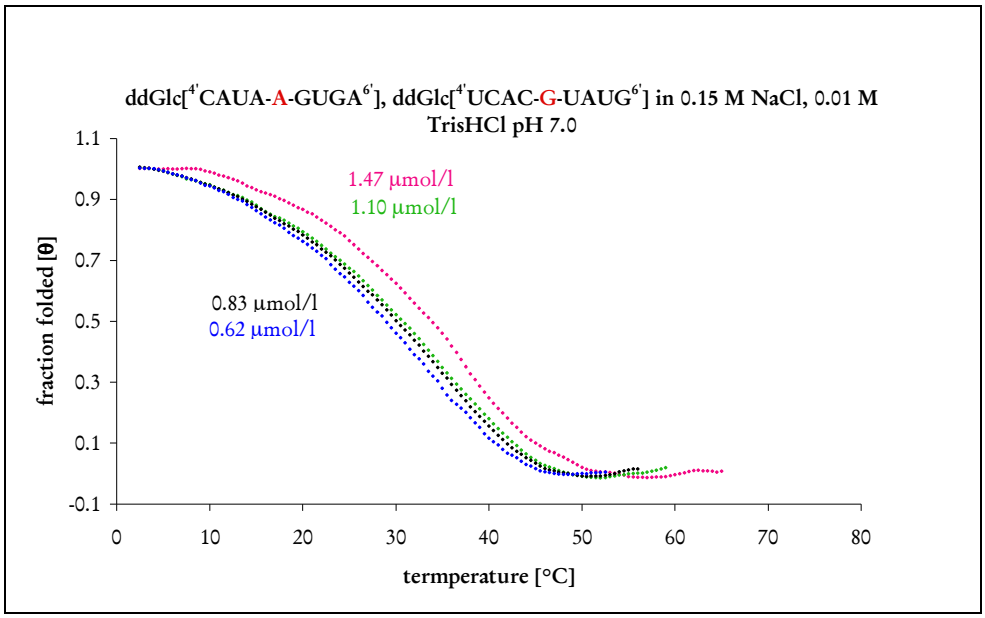
**Fig. 61** UV-melting curves (at 260 nm) of the nonanucleotides ddGlc[$^{4'}$CAUAAGUGA$^{6'}$] and ddGlc[$^{4'}$UCACCUAUG$^{6'}$] (values obtained in 0.15 M NaCl, TrisHCl pH 7.0) (top left) and linear regression $1/T_m$ vs. ln(c) resulting in *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) (bottom).

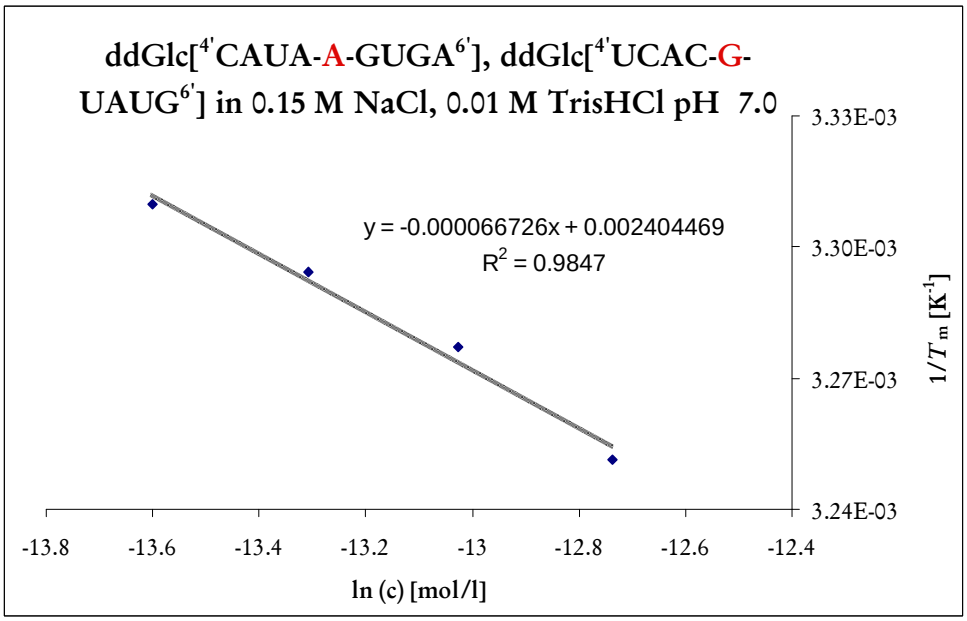





| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | $\Delta H$ [kJ/mol] | $\Delta S$ [J/(mol*K)] | $\Delta G$ (T=25 °C) [kJ/mol] |
|---|---|---|---|---|---|
| ddGlc[4'CAUADGUGA6']<br>ddGlc[4'UCACCUAUG6'] | 1.96 | 31.0 | **-126.9** | **-302.9** | **-36.6** |
| ddGlc[4'CAUADGUGA6']<br>ddGlc[4'UCACCUAUG6'] | 1.47 | 28.5 | | | |
| ddGlc[4'CAUADGUGA6']<br>ddGlc[4'UCACCUAUG6'] | 1.14 | 27.0 | | | |
| ddGlc[4'CAUADGUGA6']<br>ddGlc[4'UCACCUAUG6'] | 0.62 | 24.0 | | | |

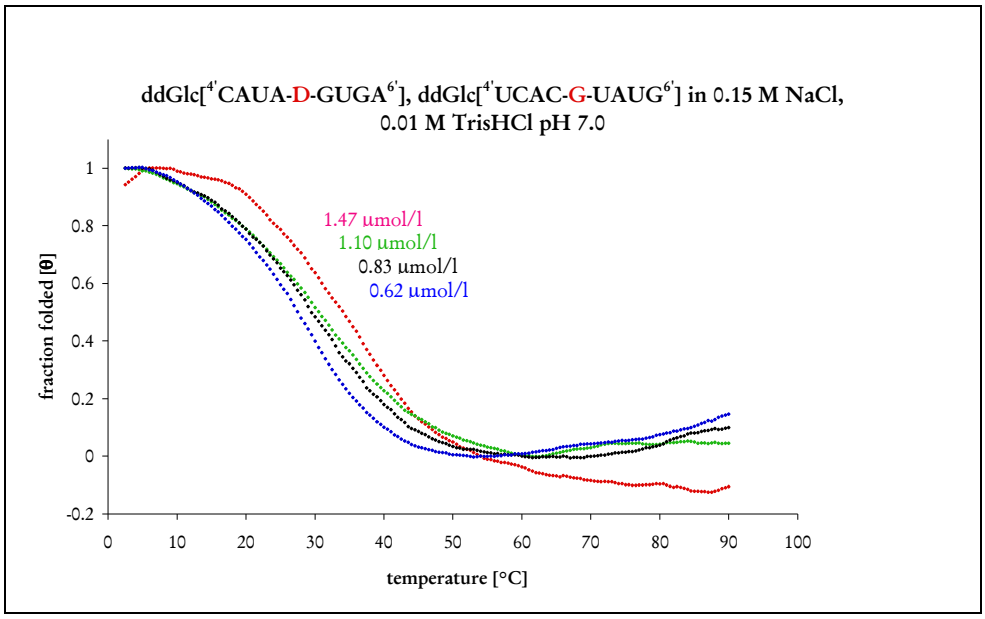
**Fig. 62** UV-melting curves (at 260 nm) of the nonanucleotides ddGlc[4'CAUADGUGA6'] and ddGlc[4'UCACCUAUG6'] (values obtained in 0.15 M NaCl, TrisHCl pH 7.0) (top left) and linear regression $1/T_m$ vs. ln(c) resulting in *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) (bottom).

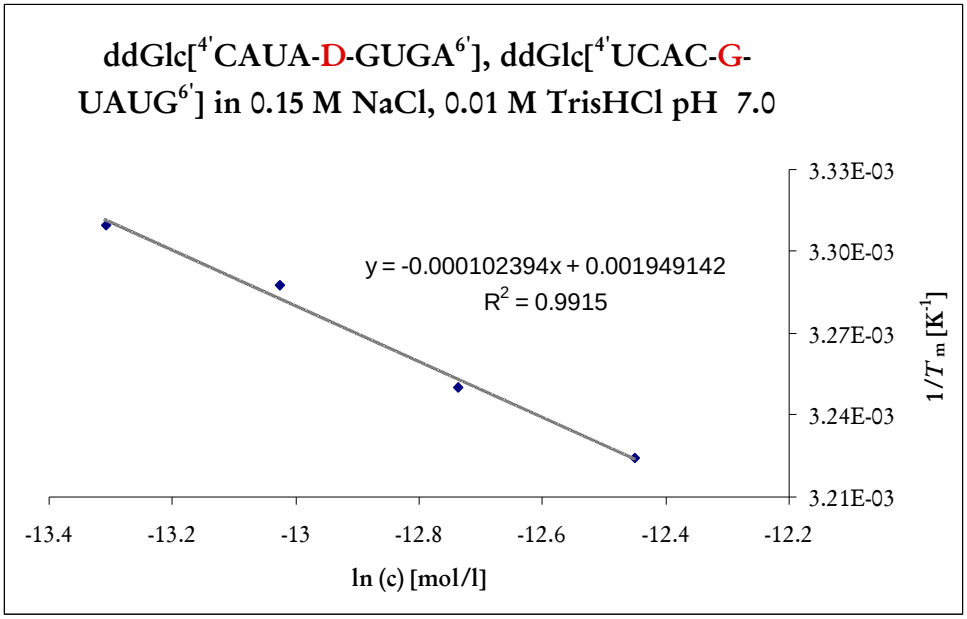

ddGlc[4'CAUA-A-GUGA6'], ddGlc[4'UCAC-G-UAUG6'] in 0.15 M NaCl, 0.01 M TrisHCl pH 7.0
1.47 µmol/l
1.10 µmol/l
0.83 µmol/l
0.62 µmol/l
fraction folded [θ]
termperature [°C]
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
0
10
20
30
40
50
60
70
80


ddGlc[4'CAUA-A-GUGA6'], ddGlc[4'UCAC-G-UAUG6'] in 0.15 M NaCl, 0.01 M TrisHCl pH 7.0
y = -0.000066726x + 0.002404469
R2 = 0.9847
1/Tm [K-1]
ln (c) [mol/l]
3.33E-03
3.30E-03
3.27E-03
3.24E-03
-13.8
-13.6
-13.4
-13.2
-13
-12.8
-12.6
-12.4

| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | $\Delta H$ [kJ/mol] | $\Delta S$ [J/(mol*K)] | $\Delta G$ (T=25 °C) [kJ/mol] |
|---|---|---|---|---|---|
| ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**G**UAUG$^{6'}$] | 1.47 | 34.4 | **-124.6** | **-288.1** | **-38.7** |
| ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**G**UAUG$^{6'}$] | 1.10 | 32.0 | | | |
| ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**G**UAUG$^{6'}$] | 0.83 | 30.4 | | | |
| ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**G**UAUG$^{6'}$] | 0.62 | 29.0 | | | |

**Fig. 63** UV-melting curves (at 260 nm) of the nonanucleotides ddGlc[$^{4'}$CAUAAGUGA$^{6'}$] and ddGlc[$^{4'}$UCACGUAUG$^{6'}$] (values obtained in 0.15 M NaCl, TrisHCl pH 7.0) (top left) and linear regression $1/T_m$ vs. ln(c) resulting in *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) (bottom).






| Oligonucleotide | Concentration [µM] | $T_m$ [°C] | $\Delta H$ [kJ/mol] | $\Delta S$ [J/(mol*K)] | $\Delta G$ (T=25 °C) [kJ/mol] |
|---|---|---|---|---|---|
| ddGlc[4'CAUADGUGA6']<br>ddGlc[4'UCACGUAUG6'] | 1.96 | 37.0 | **-81.2** | **-146.7** | **-37.2** |
| ddGlc[4'CAUADGUGA6']<br>ddGlc[4'UCACGUAUG6'] | 1.47 | 34.5 | | | |
| ddGlc[4'CAUADGUGA6']<br>ddGlc[4'UCACGUAUG6'] | 1.10 | 31.0 | | | |
| ddGlc[4'CAUADGUGA6']<br>ddGlc[4'UCACGUAUG6'] | 0.83 | 29.0 | | | |

**Fig. 64** UV-melting curves (at 260 nm) of the nonanucleotides ddGlc[4'CAUADGUGA6'] and ddGlc[4'UCACGUAUG6'] (values obtained in 0.15 M NaCl, TrisHCl pH 7.0) (top left) and linear regression $1/T_m$ vs. ln(c) resulting in *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) (bottom).

The *Van't Hoff*-pairing enthalpies ($\Delta H$) and –entropies ($\Delta S$) obtained for oligonucleotides with the general formula ddGlc[4'CAUA-**X**-GUGA6'], ddGlc[4'CAUA-**Y**-GUGA6'] are summarized in **Fig. 65**. An adenine – 2,4- diaminopyrimidine exchange resulted in all observed cases in an

enthalpically destabilization and entropically stabilization of the duplex compared to the unmodified homo-DNA oligonucleotide.

| Oligonucleotide | *ΔH* [kJ/mol] | *ΔS* [J/(mol*K)] | *ΔG* (T=25 °C) [kJ/mol] |
|---|---|---|---|
| ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**U**UAUG$^{6'}$] | **-152.5** | **-365.7** | **-43.5** |
| ddGlc[$^{4'}$CAUA**D**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**U**UAUG$^{6'}$] | **-83.3** | **-149.6** | **-38.7** |
| ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**A**UAUG$^{6'}$] | **-206.8** | **-548.7** | **-43.3** |
| ddGlc[$^{4'}$CAUA**D**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**A**UAUG$^{6'}$] | **-111.1** | **-240.9** | **-39.3** |
| ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**C**UAUG$^{6'}$] | **-448.3** | **-1342.1** | **-48.1** |
| ddGlc[$^{4'}$CAUA**D**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**C**UAUG$^{6'}$] | **-126.9** | **-302.9** | **-36.6** |
| ddGlc[$^{4'}$CAUA**A**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**G**UAUG$^{6'}$] | **-124.6** | **-288.1** | **-38.7** |
| ddGlc[$^{4'}$CAUA**D**GUGA$^{6'}$]<br>ddGlc[$^{4'}$UCAC**G**UAUG$^{6'}$] | **-81.2** | **-146.7** | **-37.2** |

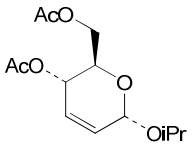
**Fig. 65** *Van't Hoff*-pairing enthalpies (*ΔH*) and –entropies (*ΔS*) of the oligonucleotides with the general formula ddGlc[$^{4'}$CAUA-X-GUGA$^{6'}$] and ddGlc[$^{4'}$UCAC-Y-UAUG$^{6'}$].

# 5. Experimental Part

1. *General*. Solvents were purified by distillation. Thin-layer chromatography (TLC): *Merck* TLC aluminum sheets, silica gel *60* $F_{254}$, 2 mm or *Machery-Nagel* Alox plastic sheets, Aluminum oxide N/$UV_{254}$, 0.2 mm. Column chromatography (CC): *Sigma-Aldrich* Silica gel Merck Type 9385, 230-400 mesh, 60 Å or *Sigma-Aldrich* aluminum oxide neutral type 507c, Brockmann I, 150 mesh inactivated with 4% $H_2O$. High-performance liquid chromatography (HPLC): *Shimadzu-10*. M. p. *Teston-DM-6300*, values are uncorrected. IR Spectra: *Perkin-Elmer-1600* FT-IR spectrophotometer; in KBr unless otherwise stated; absorption values in $cm^{-1}$ and intensity (*vs* (*very strong*, 0–20% transmission), *s* (*strong*, 20-40% transmission), *m* (*middle*, 40-60% transmission), *w* (*weak*, 60-80% transmission)). $^{1}$H-(300 MHz) and $^{13}$C-NMR (75.5 MHz) Spectra: *Bruker ARX-300* instrument; in $CDCl_3$ at 300 K unless otherwise stated; $\delta$ in ppm relative to TMS (0.00 ppm), $CHCl_3$ (7.26 ppm) or $H_3COH$ (3.31 ppm), coupling constants *J* in Hz. $^{13}$C-signal multiplicity from DEPT 90 and DEPT 135 spectra (*Disortionless Enhancement by Polarization Transfer*). Peaks assignment was performed by two-dimensional NMR experiments (COSY, HSQC, HMBC) and analogies. MS: *Finnigan MAT-95* instrument for CI and EI; *Finnigan TSQ-700* triple quadrupole spectrometer for ESI; *m/z* (rel. %); *Bruker* Autoflex I spectrometer for MALDI-TOF; Elemental Analyses were performed on a *Vario EL*-instrument (*Elementar*).

## *5.1 Synthesis*

**Isopropyl-4,6-di-*O*-acetyl-2,3-dideoxy-$\alpha,\beta$-D-erythro-hex-2-enopyranoside (20)** [57]




To a solution of tri-*O*-acetyl-D-glucal (10.0 g, 36.7 mmol) in $CH_2Cl_2$ (100 ml) was added at rt propane-2-ol (14.0 ml, 11.0 g, 0.183 mol) and dropwise $BF_3$*$OEt_2$ (1.70 ml, 1.96 g, 13.8 mmol). After stirring for 22 h at rt, the reaction was quenched by the addition of aq. $NaHCO_{3\ sat.}$ (200 ml) followed by solid $NaHCO_3$ until no further foaming occurred. The organic phase was separated and the aqueous phase was extracted with $CH_2Cl_2$ (2 x 200 ml). The combined organic phase was washed with aq. $NaCl_{sat.}$ (1 x 100 ml), dried ($Na_2SO_4$) and evaporated, yielding 9.78 g (35.9 mmol, 98%, $\alpha$-D/$\beta$-D = 9:1 (GC)) of **20** as a yellow oil.

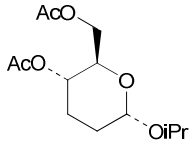
$R_f$ ($SiO_2$; $Et_2O$/hexane 1:1) 0.22 ($Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, $CDCl_3$): 5.99-5.79 (*m*, HC(3), HC(2)); 5.32-5.28 (*m*, HC(4)); 5.21 (*d*, *J* = 1.5, HC(1), $\beta$-D); 5.14 (broad *s*, HC(1), $\alpha$-D); 4.27-4.12 (*m*, $CH_2$(6), $H_3CCHCH_3$); 4.03-3.95 (*m*, HC(5)); 2.10, 2.08 (2*s*, $CH_3$(acetyl)); 1.25 (*d*, *J* = 6.3, $CH_3$($^i$Pr)); 1.18 (*d*, *J* = 6.1, $CH_3$($^i$Pr)).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 170.7, 170.2 (2*s*, 2 CO, $\alpha$-D); 131.1, 125.7 (2*d*, C(2), C(3), $\beta$-D); 128.7, 128.3 (2*d*, C(2), C(3), $\alpha$-D); 93.1 (*d*, C(1), $\beta$-D); 92.7 (*d*, C(1), $\alpha$-D); 72.5 (*d*, CH($^i$Pr), $\beta$-D); 70.6 (*d*, CH($^i$Pr), $\alpha$-D); 70.1, 64.4 (2*d*, C(4), C(5), $\beta$-D); 66.6, 65.3 (2*d*, C(4), C(5), $\alpha$-D); 63.4 (*t*, C(6), $\beta$-D); 63.0 (*t*, C(6), $\alpha$-D); 23.4, 21.9 (2*q*, $2CH_3$($^i$Pr), $\alpha$-D); 21.6 (*q*, $CH_3$($^i$Pr), $\beta$-D); 20.9, 20.7 (2*q*, $2CH_3$(acetyl), $\alpha$-D).

EI-MS: 214.1 (5, $[M\text{-}O^{i}Pr]^{+}$), 213 (13, $[M\text{-}C_2H_3O_2]^{+}$), 170 (18), 128 (100), 86 (96), 57 (14), 43 (96, $[H_3CCO]^{+}$).

**Isopropyl-4,6-di-*O*-acetyl-2,3-dideoxy-$\alpha,\beta$-D-glucopyranoside (21)**[57]




**20** (3.00 g, 11.0 mmol) was suspended with 10% Pt/C (153 mg) in EtOAc (61 ml) in a normal pressure hydrogenation apparatus. The suspension was evacuated and flushed with $H_2$ (5 times). Stirring for 15 h at rt under a $H_2$-athmosphere, filtration over celite and evaporation under reduced pressure resulted in 3.00 g (10.9 mmol, 99%, $\alpha$-D/$\beta$-D= 9:1 (GC)) of **21** as a colourless oil.

$R_f$ ($SiO_2$ ; $Et_2O$/hexane 1:1) 0.40 ($Ce(SO_4)_2$).

IR (film): 3465*m*, 2970*vs*, 2727*w*, 2474*w*, 2278*w*, 2107*w*, 2034*w*, 1937*w*, 1901*w*, 1740*vs*, 1657*m*, 1440*s*, 1371*vs*, 1330*s*, 1234*vs*, 1177*s*, 1125*vs*, 1041*vs*, 995*vs*, 948*s*, 906*w*, 855*m*, 736*m*, 702*w*, 665*m*, 647*m*, 605*m*, 584*m*, 525*m*, 490*w*.

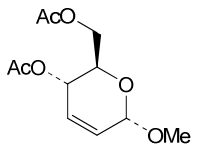
$^{1}$H-NMR (300 MHz, $CDCl_3$): 4.95 (broad *s*, HC(1)); 4.73 (*dt*, *J* = 10.1, 5.0, HC(4)); 4.26 (*dd*, *J* = 11.9, 5.2, 1H of $H_2C(6)$); 4.09 (*dd*, *J* = 11.9, 2.0, 1H of $H_2C(6)$); 4.02-3.92 (*m*, HC(5)); 3.93 ($m_c$, $H_3CC\mathit{H}CH_3$); 2.09, 2.05 (2*s*, $CH_3$(acetyl)); 2.08-1.74 (*m*, $H_2C(3)$, $H_2C(2)$) ; 1.23 (*d*, *J* = 6.3, $CH_3(^{i}Pr)$) ; 1.15 (*d*, *J* = 5.9, $CH_3(^{i}Pr)$).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 170.8, 170.0 (2*s*, 2CO, $\alpha$-D), 99.5 (*d*, C(1), $\beta$-D), 99.1 (*d*, C(1), $\alpha$-D); 74.7, 68.5 (2*d*, C(4), C(5), $\beta$-D); 68.5, 67.9 (2*d*, C(4), C(5), $\alpha$-D); 63.4 (*t*, C(6), $\beta$-D); 63.1 (*t*, C(6),

$\alpha$-D); 29.1, 23.8 (2*t*, C(2), C(3), $\alpha$-D); 23.1, 21.3 (2*q*, $2CH_3(^iPr)$, $\alpha$-D); 21.0, 20.7 (2*q*, $2CH_3$(acetyl), $\alpha$-D).

EI-MS: 215 (10, $[M-H_3CCOO]^+$), 99 (32), 86 (56), 43 (100, $[H_3CCO]^+$).

**Methyl-4,6-di-*O*-acetyl-2,3-dideoxy-$\alpha,\beta$-D-erythro-hex-2-enopyranoside (16)**[32a,55]




To a solution of 40.5 g (0.149 mol) of 3,4,6-tri-*O*-acetyl-D-glucal in 200 ml of toluene and 10 ml of MeOH at 0 °C was added 10 ml (8.70 g, 61.3 mmol) $BF_3$*$OEt_2$ dropwise over 8 min. After removing the ice bath, the reaction was stirred for 40 min. 290 ml of toluene was added, followed by $NaHCO_{3\ sat.}$ (150 ml) and further solid $NaHCO_3$ until no more foaming occurred. The org. phase was separated and the aq. phase was extracted with toluene (2 x 100 ml), the combined org. phases were dried ($MgSO_4$) and evaporated under reduced pressure. The slightly yellow oil was dissolved in 200 ml MeOH stirred for 30 min at 40° C with 1 g activated charcoal, filtered over celite and evaporated to give **16** as a colourless oil: 34.2 g (0.140 mmol, 94 %, $\alpha$-D/$\beta$-D = 8:1 (GC, $^1$H-NMR)).

$R_f$ ($SiO_2$ ; $Et_2O$/hexane 1:1) 0.27 ($Ce(SO_4)_2$).

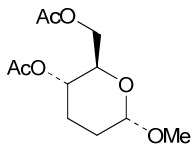
IR ($CHCl_3$): 3029*m*, 3010*m*, 2960*m*, 2935*m*, 2910*m*, 2830*w*, 1745*vs*, 1740*vs*, 1465*w*, 1450*m*, 1440*m*, 1420*m*, 1405*m*, 1395*m*, 1370*s*, 1340*w*, 1250*vs*, 1185*s*, 1150*m*, 1135*m*, 1110*m*, 1070*s*, 1050*vs*, 1020*s*, 965*s*, 910*s*, 870*w*, 830*w*, 710*w*, 660*w*, 645*w*, 605*m*.

$^1$H-NMR (300 MHz, $CDCl_3$): 6.02-5.81 (*m*, HC(2), HC(3)); 5.33 (*dd*, *J* = 9.6, 1.4, HC(4), *α*-D); 5.20 (*d*, *J* = 1.4, HC(4), *β*-D); 5.04 (*d*, *J* = 1.4, HC(1), *β*-D); 4.93 (*d*, *J* = 1.2, *α*-D); 4.29-4.16 (*m*, $H_2C$(6)); 4.11-4.05 (*m*, HC(5)); 3.47 (*s*, MeO, *β*-D); 3.45 (*s*, MeO, *α*-D); 2.11, 2.10, 2.09 (3*s*, 2 Ac).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 170.6 (*s*, CO); 170.1 (*s*, CO); 130.1, 126.2 (2*d*, C(2), C(3), *β*-D); 129.1, 127.6 (2*d*, C(2), C(3), *α*-D); 95.9 (*d*, C(1), *β*-D); 95.3 ((*d*, C(1), *α*-D); 72.7, 64.1 (2*d*, C(4), C(5), *β*-D); 66.8, 65.2 (2*d*, C(4), C(5), *α*-D); 63.3 (*t*, C(6), *β*-D); 62.9 (*t*, C(6), *α*-D); 55.8 (*q*, MeO, *α*-D); 55.2 (*q*, MeO, *β*-D); 20.8, 21.0 (2*q*, *Me*CO).

EI-MS: 213 (2), 153 (4), 142 (13), 111 (15), 100 (100), 71 (12), 43 (34, $[CH_3CO]^+$).

**Methyl-4,6-di-*O*-acetyl-2,3-dideoxy-*α,β*-D-glucopyranoside (17)**[32a,55]




To a solution of 35.1 g (0.144 mol) of **16** in 100 ml of MeOH and 1 ml of AcOH was added in a normal pressure hydrogenation apparatus 175 mg of 10% Pd(C). After twice evacuating and flashing with $H_2$, the reaction was stirred for 8 h at 25° C, filtered over celite, evaporated, dissolved in 300 ml EtOAc, extracted with $NaHCO_{3\ sat.}$ (1 x 150 ml), dried ($MgSO_4$) and evaporated under reduced pressure, resulting in 29.0 g (0.118 mol, 82 %, *α*-D/*β*-D = 8:1 (GC, $^1$H-NMR)) **17** as a slightly yellow oil.

$R_f$ ($SiO_2$; $Et_2O$/hexane 1:2) 0.35 ($Ce(SO_4)_2$).

IR ($CHCl_3$): 3021*m*, 2950*m*, 2909*w*, 2853*w*, 2828*w*, 1734*vs*, 1455*m*, 1370*m*, 1302*w*, 1245*vs*, 1235*vs*, 1180*w*, 1130*m*, 1105*m*, 1080*m*, 1063*m*, 1051*s*, 1040*s*, 996*m*, 976*m*, 959*m*, 925*w*, 897*w*, 884*w*, 870*w*, 858*w*, 844*w*, 660*w*.

$^1$H-NMR (300 MHz, $CDCl_3$): 4.77-4.63 (*m*, HC(4), *β*-D; HC(1), HC(4), *α*-D); 4.44 (*dd*, *J* = 8.5, 2.0, HC(1), *β*-D); 4.28 (*dd*, *J* = 12.0, 5.2, 1H $H_2C$(6), *β*-D); 4.26 (*dd*, *J* = 12.0, 5.3, 1H $H_2C$(6), *α*-D); 4.21-4.16 (*m*, 1H $H_2C$(6), *β*-D); 4.11 (*dd*, *J* = 12.0, 2.3, 1H $H_2C$(6), *α*-D); 4.03-3.96 (*m*, HC(5), *β*-D); 3.90 (*ddd*, *J* = 10.0, 5.3, 2.3, HC(5), *α*-D); 3.49 (*s*, MeO, *β*-D); 3.36 (*s*, MeO, *α*-D); 2.09, 2.05 (2*s*, 2 Ac).

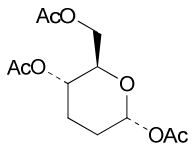
$^{13}$C-NMR (75 MHz, $CDCl_3$): 170.8, 169.9 (2*s*, 2CO); 102.5 (*d*, C(1), *β*-D); 97.4 (*d*, C(1), *α*-D); 68.6, 67.7 (2*d*, C(4), C(5), *α*-D); 68.1, 67.5 (2*d*, C(4), C(5), *β*-D); 63.3 (*t*, C(6), *β*-D); 63.1 (*t*, C(6), *α*-D); 56.4 (*q*, MeO, *β*-D); 54.5 (*q*, MeO, *α*-D); 29.3, 24.9 (2*t*, C(2), C(3), *β*-D); 28.7, 24.0 (2*t*, C(2), C(3), *α*-D); 20.9, 20.7 (2*q*, 2 MeCO).

EI-MS: 215 (14), 144 (14), 101 (21), 84 (38), 58 (100), 43 (88, $[CH_3CO]^+$).

ESI-MS: 269.1 (100, [m+Na]).

HR-ESI-MS: calc. for $C_{11}H_{18}O_6Na_1$ ($[m+Na]^+$) 269.1000; found 269.1001

**Acetyl-4,6-di-*O*-acetyl-2,3-dideoxy-*α,β*-D-glucopyranoside (19)**[57]



To a solution of **17** (2.00 g, 8.12 mmol) in 14 ml of AcOH was added 3.5 ml (3.80 g, 37.3 mmol) of $Ac_2O$ at 0 °C, followed by 0.4 ml of conc. $H_2SO_4$. After removal of the ice bath, the reaction was stirred for 6 min. The yellow solution was poured onto 200 ml of ice, extracted with $CH_2Cl_2$ (2 x 100 ml), and the org. phase was washed with $H_2O$ (1 x 100 ml) and $NaHCO_{3\ sat.}$ (1 x 100 ml), dried ($Na_2SO_4$) and evaporated, yielding 1.67 g (6.09 mmol, 76 %, $\alpha$-D/$\beta$-D = 5:1 (GC)) of **19** as a colourless oil.

$R_f$ ($SiO_2$; $Et_2O$/hexane 1:1) 0.24 ($Ce(SO_4)_2$).

IR (film) 3650*w*, 3467*w*, 2960*m*, 1744*vs* (br.), 1655*w*, 1439*m*, 1369*s*, 1241*vs*, 1132*s*, 1087*s*, 1044*vs*, 1004*s*, 953*s*, 918*s*, 735*w*, 605*m*, 540*w*.

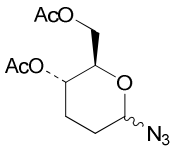
$^1$H-NMR (300 MHz, $CDCl_3$): 6.13 (broad *s*, HC(1), $\alpha$-D); 5.78 (*dd*, *J* = 8.3, 2.4, HC(1), $\beta$-D); 4.80 (*dt*, *J* = 10.0, 4.5, HC(4), $\alpha$-D); 4.26 (*dd*, *J* = 12.0, 4.6, 1H $H_2C$(6), $\alpha$-D); 4.10 (*dd*, *J* = 12.0, 2.0, 1H $H_2C$(6), $\alpha$-D); 4.02 (*ddd*, *J* = 10.0, 4.6, 2.0, HC(5), $\alpha$-D); 2.12, 2.08, 2.06 (3*s*, 3 Ac); 1.96-1.64 (*m*, $H_2C$(3), $H_2C$(2)).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 170.7, 169,7, 169.1 (3*s*, 3CO); 93.2 (*d*, C(1), $\beta$-D); 90.7 (*d*, C(1), $\alpha$-D); 75.4, 66.4 (2*d*, C(4), C(5), $\beta$-D); 70.7, 67.0 (2*d*, C(4), C(5), $\alpha$-D); 62.8 (*t*, C(6), $\beta$-D); 62.6 (*t*, C(6), $\alpha$-D); 27.7, 25.6 (2*t*, C(2), C(3), $\beta$-D); 27.5, 23.5 (2*t*, C(2), C(3), $\alpha$-D); 20.9, 20.8, 20.6 (3*q*, 3 MeCO).

EI-MS: 215 (20, $[M\text{-}CH_3CO]^+$), 155 (9), 145 (48), 103 (27), 94 (52), 69 (44), 43 (100, $[CH_3CO]^+$).

HR-ESI-MS: calc. for $C_{12}H_{18}O_7Na_1$ ($[m+Na]^+$) 297.0950; found 297.0944.

**Azido-4,6-di-*O*-acetyl-2,3-dideoxy-$\alpha,\beta$-D-glucopyranoside (81)**




To a solution of 5.36 g (19.5 mmol) **19** in 210 ml of $CH_2Cl_2$ was added dropwise 5.2 ml (4.55 g, 39.5 mmol) of azidotrimethylsilane, followed by 4.7 ml (5.76 g, 25.9 mmol) trimethylsilyl triflate. After stirring for 30 min at rt, the reaction mixture was cooled to 0°C and poured on water/ice (100 ml). The organic phase was separated and washed with 1 M HCl (1 x 40 ml), $H_2O$ (1 x 40 ml), aq. $NaCl_{sat.}$ (1 x 40 ml), aq. $NaHCO_{3\ sat.}$ (1 x 40 ml), aq. $NaCl_{sat.}$ (1 x 40 ml), dried ($Na_2SO_4$) and evaporated. CC (200 g $SiO_2$, $Et_2O$/hexane 1:2, 2% $NEt_3$) yielded 4.56 g (17.7 mmol, 91 %, ($\alpha$-D/$\beta$-D= 1:1 (GC, $^1$H-NMR) of **81** as a slightly yellow oil.

$R_f$ ($SiO_2$; $Et_2O$/hexane 1:1) 0.42 ($Ce(SO_4)_2$).
$R_f$ ($SiO_2$; $Et_2O$/hexane 1:2) 0.20 ($Ce(SO_4)_2$).

IR (film): 3637*w*, 3466*w*, 3326*w*, 2958*vs*, 2878*s*, 2728*w*, 2463*w*, 2112*vs* ($N_3$), 1742*vs*, 1742*vs*, 1439*vs*, 1369*vs*, 1235*vs*, 1042*vs*, 1000*vs*, 973*s*, 947*s*, 905*vs*, 858*vs*, 739*w*, 706*w*, 648*m*, 626*m*, 605*s*, 568*m*, 547*w*, 529*w*.

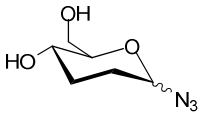
$^1$H-NMR (300 MHz, $CDCl_3$): 5.42 (*d*-like, HC(1), $\alpha$-D); 4.77-4.67 (*m*, HC(4), $\alpha$-D, $\beta$-D, HC(1), $\beta$-D); 4.31-4.14 (*m*, $H_2C(6)$, $\alpha$-D, $\beta$-D); 4.06-4.00, 3.74-3.68 (2*m*, HC(5), $\alpha$-D, $\beta$-D); 2.09, 2.07, 2.06 (3*s*, 2CH3, $\alpha$-D, $\beta$-D); 2.28-2.21, 1.93-1.56 (2*m*, $H_2C(3)$, $H_2C(2)$, $\alpha$-D, $\beta$-D).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 172.3, 171.4 (2*s*, 2CO); 89.8 (*d*, C(1), $\beta$-D); 88.1 (*d*, C(1), $\alpha$-D); 78.0, 72.3, 68.7, 68.3 (4*d*, C(5), C(4), $\alpha$-D, $\beta$-D); 64.4, 64.2 (2*t*, C(6), $\alpha$-D, $\beta$-D); 30.8, 29.7, 28.7, 25.3 (4*t*, C(3), C(2), $\alpha$-D, $\beta$-D); 22.5, 22.3 (2*q*, 2 MeCO).

EI-MS: 215 (20, $[M\text{-}N_3]^+$), 155 (11), 113 (6), 95 (12), 67 (8), 43 (100, $[CH_3CO]^+$).

ESI-MS: 280 ($[M+Na]^+$).

**Azido-2,3-dideoxy-$\alpha,\beta$-D-glucopyranoside (82)**




To a solution of **81** (1.00 g, 3.89 mmol) dissolved in MeOH (8 ml) were added 3.3 g of amberlite IRA 402 (washed with 50 ml 2 N aq. NaOH, 50 ml $H_2O$, 50 ml MeOH) in one portion at rt. The reaction was stirred for 40 min at rt, filtered, evaporated under reduced pressure, yielding 612 mg (3.53 mmol, 91 %, $\alpha$-D/$\beta$-D 1:1 ($^1$H-NMR)) of **82** as highly viscous, colourless oil.

$R_f$ ($SiO_2$; $CH_2Cl_2$/MeOH 5 :1) 0.56 ($Ce(SO_4)_2$).

IR (film): 3374*s*, 2937*s*, 2874*m*, 2467*w*, 2109*vs*, 2044*w*, 1719*w*, 1661*m*, 1596*w*, 1456*m*, 1440*m*, 1415*m*, 1373*m*, 1328*m*, 1240*s*, 1174*w*, 1100*s*, 1048*vs*, 1001*m*, 975*s*, 931*m*, 886*w*, 865*m*, 843*w*, 831*w*, 736*m*, 702*w*, 664*w*, 626*m*, 568*w*.

$^1$H-NMR (300 MHz, d$^4$-MeOD): 5.42 (br. *s*, HC(1), $\alpha$-D); 4.75-4.69 (*m*, HC(1), $\beta$-D); 3.89-3.77, 3.72-3.65, 3.62-3.55, 3.52-3.38, 3.35-3.28 (5*m*, HC(4), HC(5), $H_2C(6)$, $\alpha$-D/$\beta$-D); 2.12-2.01, 1.91-1.48 (2*m*, $H_2C(2)$, $H_2C(3)$, $\alpha$-D/$\beta$-D).

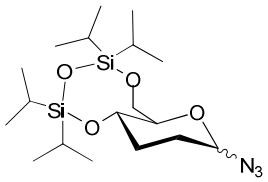
$^{13}$C-NMR (75 MHz, d$^4$-MeOD): 89.2 (*d*, C(1), $\beta$-D); 87.8 (*d*, C(1), $\alpha$-D); 82.9, 77.0, 65.9, 65.8 (4*d*, C(4), C(5), $\alpha$-D, $\beta$-D); 62.5, 62.3 (2*t*, C(6), $\alpha$-D, $\beta$-D); 31.4, 30.7, 29.3, 27.4 (4*t*, C(2), C(3), $\alpha$-D, $\beta$-D).

CI-MS: 191.4 (49, $[m+NH_4]^+$), 163.1 (21), 148.1 (100), 146.1 (16), 131.1 (72, $[m-N_3]^+$), 112.1 (10), 110.1 (17), 98.1 (8).

ESI-MS: 196.0 (100, $[m+Na]^+$), 185.0 (21), 155.0 (19).

HR-ESI-MS: calc. for $C_6H_{11}N_3O_3Na_1$ ($[m+Na]^+$) 196.0698; found 196.0695.

**Azido-4,6-di-*O*-(tetraisopropyldisiloxane)-2,3-dideoxy-$\alpha,\beta$-D-glucopyranoside (83)**




After drying for 4 h under high vacuum ($p < 10^{-1}$ mbar) the diol **82** (502 mg, 2.90 mmol) was dissolved under sonication in 11.5 ml DMF. Imidazole (869 mg, 12.76 mmol) was added to the solution at rt followed by a dropwise addition of tetraisopropyldichlorosiloxane (1.0 ml, 3.19 mmol) to the rapidly stirring solution within 15 min. The colourless solution was stirred at rt for 4 h, and 1.7 ml $CH_3OH$ was added and the reaction was extracted with EtOAc (3 x 25 ml), the combined org. phases were dried ($Na_2SO_4$) and evaporated under reduced pressure. CC (20 g $SiO_2$, $Et_2O$/hexane 1:10, application of the compound with $CH_2Cl_2$) yielded 1.05 g (2.53 mmol, 87%, $\alpha$-D/$\beta$-D 1:1 ($^1$H-NMR)) of **83** as a colourless oil.

$R_f$ ($SiO_2$; $Et_2O$/hexane 1:1) 0.70 ($Ce(SO_4)_2$).

$R_f$ ($SiO_2$; $Et_2O$/hexane 1:10) 0.31 ($Ce(SO_4)_2$).

IR (film): 3325*w*, 2945*vs*, 2894*m*, 2868*vs*, 2725*w*, 2111*vs*, 1465*s*, 1439*w*, 1387*m*, 1325*w*, 1307*w*, 1292*w*, 1264*m*, 1247*m*, 1182*w*, 1145*m*, 1091*vs*, 1035*vs*, 996*vs*, 943*w*, 919*w*, 885*s*, 861*m*, 846*m*, 821*w*, 773*w*, 739*m*, 701*w*, 622*w*, 595*w*, 559*w*.

$^{1}$H-NMR (300 MHz, $CDCl_3$): 5.42 (br. *s*., HC(1), *α*-D); 4.64 (*dd*, *J* = 10.1, 2.3, HC(1), *β*-D); 4.17 (*dd*, *J* = 12.5, 2.0, 1H of $H_2C(6)$); 4.12 (*dd*, *J* = 12.5, 2.2, 1H of $H_2C(6)$); 3.95-3.80 (*m*, 4H of HC(4), HC(5), $H_2C(6)$); 3.63-3.57 (*m*, 1H of HC(4), HC(5), $H_2C(6)$); 3.26-3.19 (*m*, 1H of HC(4), HC(5), $H_2C(6)$); 2.13-2.04, 1.92-1.52 (2*m*, $H_2C(2)$, $H_2C(3)$); 1.21-0.87 (*m*, 2 x 28H of Si*CH*($CH_3$)$_2$).

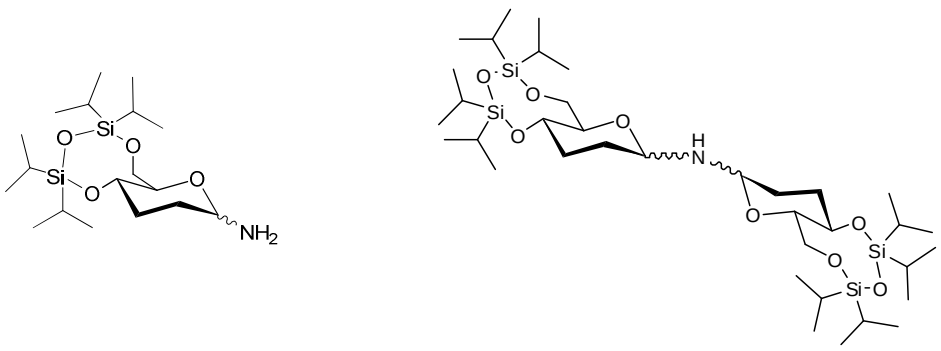
$^{13}$C-NMR (75 MHz, $CDCl_3$): 89.2 (*d*, C(1), *β*-D); 87.3 (*d*, C(1), *α*-D); 82.0, 76.1, 64.8, 64.3 (4*d*, C(4), C(5), *α*-D, *β*-D); 64.8, 64.3 (2*t*, C(6), *α*-D, *β*-D); 31.0, 30.4, 29.3, 27.3 (4*t*, C(2), C(3), *α*-D, *β*-D);17.4, 17.3, 17.21, 17.19 (4*q*, $H_3C$, *α*-D, *β*-D); 13.5, 13.3, 12.6, 12.5 (4*d*, Si*C*H).

CI-MS: 433.3 (26, $[m+NH_4]^+$), 405.3 (23), 390.3 (51), 373.3 (100, $[m-N_3]^+$), 329.2(17), 278.2 (12), 95.1 (15).

ESI-MS: 438.3 (100, $[m+Na]^+$), 413.4 (10), 397.3 (8).

HR-ESI-MS: calc. for $C_{18}H_{37}N_3O_4Na_1Si_2$ ($[m+Na]^+$) 438.2220; found 438.2225.

**Amino-4,6-di-*O*-(tetraisopropyldisiloxane)-2,3-dideoxy-*α,β*-D-glucopyranoside (84)**



To a solution of 400 mg (0.963 mmol) **83** in 8 ml of EtOH was added 180 mg of *Lindlar*'s catalyst at rt. After twice evacuating and flashing with $H_2$ in a *Parr*-apartus (20 psi), the reaction was shaken for 2 h at 25 °C, filtered over celite, and evaporated under reduced pressure, resulting in 375 mg (0.962 mmol, 99 %) of **84** as a colourless foam.

M.P. 116 °C

$R_f$ ($SiO_2$; $Et_2O$/hexane 1:2) 0.11 ($Ce(SO_4)_2$).

IR (KBr): 3425br. *s*, 2945*vs*, 2894*s*, 2868*vs*, 1633*w*, 1496*w*, 1466*s*, 1386*m*, 1366*w*, 1311*w*, 1294*m*, 1249*m*, 1219*w*, 1174*m*, 1142*w*, 1090*v*s, 1033*vs*, 1011*vs*, 991*s*, 974*s*, 934*m*, 918*w*, 902*m*, 885*vs*, 844*s*, 775*w*, 699*s*, 661*m*, 594*w*, 554*w*.

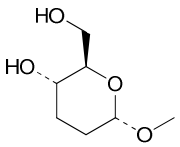
$^1$H-NMR (300 MHz, $CDCl_3$): 4.34 (*dd*, *J* = 10.1, 1.5, 1H); 4.22-4.04 (*m*, 1H); 4.10 (*dd*, *J* = 12.2, 2.2, 1H); 3.88-3.68 (*m*, 4H); 3.14 (*dt*-like, 1H); 2.08-1.32 (*m*, $H_2C(2)$, $H_2C(3)$, 8H); 1.11-0.95 (*m*, 2 x 28H of $SiCH(CH_3)_2$).

CI-MS: 762.6 (58, $[2m+H]^+$), 744.6 (100), 390.3 (32, $[m+H]^+$), 373.3 (53, $[m-NH_2]^+$), 346.3 (15), 329.2 (8), 278.3 (18).

ESI-MS: 784.6 (33, $[2m-NH_3+Na]^+$), 762.5 (32, $[2m-NH_3+H]^+$), 405.3 (100, $[m-NH_2+HOCH_3]^+$), 390.3 (37, $[m+H]^+$), 373.3 (67, $[m-NH_2]^+$).

HR-ESI-MS: calc. for $C_{36}H_{75}NO_8Na_1Si_4$ ($[2m-NH_3+Na]^+$) 784.4467; found 784.4476.

**Methyl-2,3-dideoxy-$\alpha$-D-glucopyranoside (18)**[32a,55]



To a solution of **17** (8.00 g, 32.5 mmol) dissolved in MeOH (60 ml) was added 26 g amberlite IRA 400 (washed with 300 ml 2 N aq. NaOH, 300 ml $H_2O$, 200 ml MeOH) in one portion at rt. The reaction was stirred for 2 h at rt, filtered, and evaporated under reduced pressure, yielding 5.02 g (31.0 mmol, 95 %) of **18** as a highly viscous colourless oil.

$R_f$ ($SiO_2$; $Et_2O$) 0.09 ($Ce(SO_4)_2$).

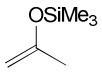
IR (KBr) : 3195*vs* (br.), 2947*s*, 1651*w*, 1565*m*, 1404*vs*, 1271*w*, 1220*w*, 1209*w*, 1171*w*, 1129*s*, 1075*m*, 1048*vs*, 1002*w*, 976*m*, 946*w*, 907*w*, 885*w*, 869*w*, 655*w*, 621*w*, 577*w*, 514*w*.

$^1$H-NMR (300 MHz, d$^4$-MeOD): 4.65 (br. *s*, HC(1), *α*-D); 4.44 (*dd*, *J* = 8.7, 2.2, HC(1), *β*-D); 3.87-3.84 (*m*, 1H $H_2C$(6), *β*-D); 3.79 (*dd*, *J* = 11.8, 2.2, 1H $H_2C$(6), *α*-D); 3.65 (*dd*, *J* = 11.7, 5.4, 1H $H_2C$(6), *α*-D); 3.50-3.43 (*m*, HC (5), HC(4), *α*–D ; HC (5), HC(4), *β*-D); 3.35 (*s*, MeO, *α*-D); 1.84-1.64 (*m*, HC(2), HC(3), *α*-D ; HC(2), HC(3), *β*-D).

$^{13}$C-NMR (75 MHz, d$^4$-MeOD): 98.5 (*d*, C(1), *α*-D); 75.1, 67.0 (2*d*, C(4), C(5), *α*-D); 54.6 (*t*, C(6), *α*-D); 30.1, 28.1 (2*t*, C(2), C(3), *α*-D).

EI-MS: 131 (83, $[M\text{-}OMe]^+$), 113 (13), 102 (93), 101 (100), 99 (78), 87 (22), 83 (15), 74 (30), 71 (73), 69 (70), 67 (23), 61 (31).

**[(1-methylethenyl)oxy]trimethyl silane**

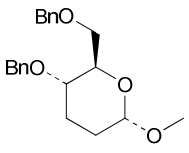



To 3.7 ml (2.92 g, 50.3 mmol) of acetone was added 8.7 ml (6.33 g, 62.6 mmol) of $NEt_3$ and stirred for 15 min at rt. 7.8 ml (9.80 g, 90.2 mmol) of freshly distilled (bulb-to-bulb-distillation) $Me_3SiCl$ was added dropwise, followed by a dropwise addition of a solution of 9.30 g (62.0 mmol) NaI in 62 ml $CH_3CN$. The reaction mixture was stirred for 25 min at rt. 50 ml pentane, followed by 30 ml of ice/water, was added and the org. phase was separated. The aq. phase was extracted with pentane (2 x 30 ml) and the combined org. phases were washed with $H_2O$ (1 x 40 ml), dried ($Na_2SO_4$) and evaporated (until $t$ = 35° C, $p$ = 400 mbar), resulting in 5.12 g (39.3 mmol, 78%) of silylenolether as a colourless liquid.

$^1$H-NMR (300 MHz, $CDCl_3$): 4.07-4.04 (*m*, $H_2C$=C); 1.78 (*dd*, *J* = 0.9, 0.4, $CCH_3$); 0.21 (*s*, Si*Me*$_3$).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 154.0 (*s*, $H_2C$=*C*); 89.3 (*t*, $H_2C$=C); 20.9 (*q*, C*C*$H_3$); 2.0 (*q*, Si*Me*$_3$).

**Methyl-4,6-di-*O*-benzyl-2,3-dideoxy-$\alpha$-D-glucopyranoside (23)**




To a suspension of **18** (2.17 g, 13.4 mmol) in 50 ml of THF at 0 °C was added over 1 h NaH (1.30 g, 50% in oil, 27.1 mmol) as solid in small portions. After stirring for 30 min at rt, $Bu_4NBr$ (50 mg, 0.135 mmol) was added, followed by BnBr (3.2 ml, 26.9 mmol). After stirring for 2 h at rt,

the reaction was poured into 200 ml aq. $NaHCO_{3\ sat.}$ and extracted with $Et_2O$ (3 x 100 ml). The combined org. phases were washed with $H_2O$ (2 x 100 ml), dried ($Na_2SO_4$) and evaporated. CC (300 g $SiO_2$, $Et_2O$: hexane 1:1) yielded 3.94 g (11.5 mmol, 86%, $\alpha$-D/$\beta$-D = 7:1 (GC)) of **23** as a slightly yellow oil.

$R_f$ ($SiO_2$; $Et_2O$/hexane 1:1) 0.38 ($Ce(SO_4)_2$).
$R_f$ ($SiO_2$; EtOAc/hexane 3:2) 0.70 ($Ce(SO_4)_2$).

IR (film): 3087*w*, 3063*m*, 3030*m*, 2935*s*, 2896*s*, 1952*w*, 1873*w*, 1811*w*, 1738*w*, 1604*w*, 1586*w*, 1496*m*, 1453*s*, 1369*s*, 1325*m*, 1261*m*, 1222*s*, 1206*s*, 1177*m*, 1100 br. *vs*, 1027*s*, 959*s*, 909*m*, 887*w*, 869*w*, 736*vs*, 697*vs*, 612*w*, 569*w*, 522*w*.

$^1$H-NMR (300 MHz, $CDCl_3$): 7.37-7.21 (*m*, 10 arom. H); 4.72 (*dd*-like, HC(1), $\alpha$-D); 4.67-4.38 (*m*, benz. H); 3.79-3.67, 3.57-3.52 (2*m*, HC(4), HC(5), $H_2C(6)$); 3.49 (*s*, MeO, 0.45H, $\beta$-D); 3.35 (*s*, MeO, 3.00H, $\alpha$-D); 2.04-2.00 (*m*, 1H of $H_2C(3)$); 1.86-1.70 (*m*, 1H of $H_2C(3)$, $H_2C(2)$).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 138.5, 138.4 (2*s*, 2 arom. $C_{quart.}$); 128.2, 127.7, 127.5, 127.43, 127.39 (5*d*, arom. C); 102.5 (*d*, HC(1), $\beta$-D); 97.4 (*d*, HC(1), $\alpha$-D); 73.4, 70.6, 69.3 (3*t*, C(6), benz. C); 72.8, 71.2 (2*d*, C(4), C(5)); 56.2 (*q*, MeO, $\beta$-D); 54.3 (*q*, MeO, $\alpha$-D); 29.7, 27.1 (2*t*, C(2), C(3)); 28.8, 23.9 (2*t*, C(2), C(3)).

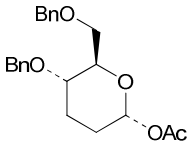
EI-MS: 113 (6), 97 (7), 91 (100), 81 (17), 65 (10), 51 (3).

ESI-MS: 367 (3, $[M+Na+2H]^+$), 366 (23, $[M+Na+H]^+$), 365 (100, $[M+Na]^+$).

HR-ESI-MS: calc. for $C_{21}H_{26}O_4Na_1$ ($[m+Na]^+$) 365.1729; found 365.1726.

Anal. calc. for $C_{21}H_{26}O_4$ (342.43) : C 73.60, H 7.66; found C 73.62, H 7.41.

**Acetyl-di-*O*-benzyl-2,3-dideoxy-$\alpha,\beta$-D-glucopyranoside (24)**




To a solution of 980 mg (2.86 mmol) **23** in 4.5 ml of AcOH at 0 °C was added, under vigorous stirring, 1.1 ml (1.20 g, 11.71 mmol) $Ac_2O$ followed by 130 µl (239 mg, 2.44 mmol) conc. $H_2SO_4$, the ice bath was removed and the yellow-brown solution stirred for 7 min at rt, poured on 5 ml ice water and solid $NaHCO_3$ was added until no more foaming occurred. Extraction with $Et_2O$ (2 x 40 ml), $H_2O$ (2 x 20 ml) and aq. $NaHCO_{3\ sat.}$ (2 x 20 ml) followed by drying of the combined org. phases ($Na_2SO_4$) and evaporation resulted, after CC (34 g $SiO_2$, $Et_2O$/hexane 1:1), in 304 mg (0.821 mmol, 31%) of **24** as a highly viscous colourless oil.

$R_f$ ($SiO_2$; $Et_2O$/hexane 1:1) 0.17 ($Ce(SO_4)_2$).

IR(film): 3088*m*, 3063*s*, 3030*s*, 3006*m*, 2937*vs*, 2867*vs*, 1954*w*, 1877*w*, 1809*w*, 1747*vs* (C=O), 1605*w*, 1586*w*, 1469*s*, 1454*vs*, 1366*vs*, 1326*m*, 1310*m*, 1244*vs*, 1215*vs*, 1184*vs*, 1096br. *vs*, 1028*vs*, 1005*vs*, 947*vs*, 905*s*, 876*s*, 820*w*, 737*vs*, 698*vs*, 606*m*, 535*w*, 462*w*.

$^1$H-NMR (300 MHz, $CDCl_3$): 7.33-7.22 (*m*, arom H); 6.16-6.12 (br. *s*, HC(1), $\alpha$-D); 4.67-4.38 (*m*, benz. H); 3.85 (*ddd*, *J* = 9.5, 3.6, 2.0, 1H of HC(4), HC(5), $H_2C(6)$); 3.79-3.36 (*m*, 3 H of HC(4), HC(5), $H_2C(6)$); 2.34-2.20, 2.13-2.04, 1.89-1.52 (3*m*, $H_2C(3)$, $H_2C(4)$; 2.08 (*s*, Ac, $\beta$-D); 2.07 (*s*, Ac, $\alpha$-D).

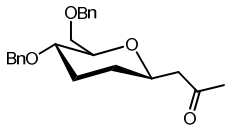
$^{13}$C-NMR (75 MHz, $CDCl_3$): 169.4 (*s*, CO); 138.4, 138.2 (2*s*, arom. $C_{quart.}$); 128.3, 128.2, 127.8, 127.63, 127.60, 127.5 (6*d*, arom. C); 91.4 (*d*, HC(1), *α*-D); 73.8, 72.1 (2*d*, C(4), C(5)); 73.4, 71.0, 68.9 (3*t*, C(6), benz. C); 27.8, 23.6 (2*t*, C(2), C(3)); 21.1 (*q*, Ac).

EI-MS: 116 (11), 98 (24), 91 (100, $[PhCH_2]^+$), 71 (97, $[C_6H_5]^+$), 65 (19), 55 (5), 51 (6).

ESI-MS: 393 (100, $[M+Na]^+$).

HR-ESI-MS: calc. for $C_{22}H_{26}O_5Na_1$ ($[m+Na]^+$) 393.1678; found 393.1678.

**1-(4',6'-di-*O*-benzyl-2',3'-dideoxy-*β*-D-glucopyranosyl)-propan-2-one (27*β*)**




Freshly distilled $SnCl_4$ (5.70 ml, 12.637 g, 48.51 mmol) was added dropwise under Ar at rt to a well stirred solution of **23** (12.002 g, 35.05 mmol), silylenolether of acetone (5.920 g, 45.45 mmol) in 600 ml dry $CH_3CN$. After stirring for 6h at rt, the reaction was carefully poured into an ice-cold solution of 250 ml aq. $NaHCO_{3\ sat.}$. 400 ml $CH_3CN$ were distilled out of the mixture. Extraction with $Et_2O$ (3 x 200 ml), followed by drying under high vacuum ($1.2 * 10^{-1}$ mbar) for several hours, and CC (300 g $SiO_2$, $Et_2O$/hexane 1:1) yielded 5.113 g (13.88 mmol) *β*-D-anomer of **27**, 0.877 g (2.38 mmol) *β*-D-, *α*-D- mixture of **27**, and 0.541 g (1.47 mmol) *α*-D-anomer **27** as slightly yellow oils (total yield 51%).

$R_f$ ($SiO_2$ ; $Et_2O$/hexane 2:1) 0.22 ($Ce(SO_4)_2$).

IR (film): 3410*w*, 3087*w*, 3062*m*, 3030*m*, 2934*s*, 2864*vs*, 1954*w*, 1876*w*, 1812*w*, 1714*vs* (CO), 1605*w*, 1586*w*, 1496*m*, 1453*s*, 1437*m*, 1418*m*, 1367*s*, 1330*m*, 1315*m*, 1285*m*, 1206*m*, 1161*s*, 1091br. *vs*, 1028*s*, 999*s*, 931*w*, 908*m*, 874*w*, 818*w*, 846*w*, 737*vs*, 698*vs*, 649*w*, 609*w*, 566*w*, 466*w*.

$^{1}$H-NMR (600 MHz, $CDCl_3$): 7.34-7.21 (*m*, arom. H); 4.63-4.39 (*m*, benz. H); 3.78 (*dddd*, *J* = 12.9, 7.4, 5.6, 2.0, HC(1)); 3.71 (*dd*, *J* = 10.7, 1.2, 1H from $H_2C(6)$); 3.67 (*dd*, *J* = 10.7, 4.4, 1H from $H_2C(6)$); 3.42-3.39 (*m*, HC(4), HC(5)); 2.75 (*dd*, *J* = 15.9, 7.1, 1H from C(1)C$H_2$CO); 2.46 (*dd*, *J* = 15.9, 5.6, 1H from C(1)C$H_2$CO); 2.29-2.23 (*m*, $HC(3)_{eq}$); 1.83-1.76 (*m*, $HC(2)_{eq}$); 1.56-1.43 (*m*, $HC(3)_{ax}$); 1.38-1.32 (*m*, $HC(2)_{ax}$).

$^{13}$C-NMR: (75 MHz, $CDCl_3$): 207.0 (*s*, CO); 138.4, 138.3 (2*s*, arom. $C_{quart.}$); 128.23, 128.19, 127.7, 127.6, 127.5, 127.4 (6*d*, arom. C); 80.5, 73,8, 72.9 (3*d*, C(1), C(4), C(5)); 73.3, 70.9, 69.6 (3*t*, 2 O$CH_2$-arom., C(6)); 49.4 (*t*, C(1)$CH_2$CO); 30.9 (*q*, Me); 30.5, 29.1 (2*t*, C(2), C(3)).

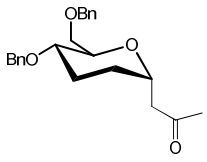
CI-MS: 386 (100, $[M+NH_4]^+$), 369 (15, $[M+H]^+$), 277 (9, $[M-C_7H_7]^+$), 206 (7), 189 (5), 91 (6, $[C_7H_7]^+$).

ESI-MS: 391.2 (100 $[M+Na]^+$).

HR-ESI-MS: calc. for $C_{23}H_{28}O_4Na_1$ ($[m+Na]^+$) 391.1885; found 391.1890.

Anal. calc. for $C_{23}H_{28}O_4$ (368.47) : C 74.97, H 7.66; found C 74.95, H 7.62.

**1-(4',6'-di-*O*-benzyl-2',3'-dideoxy-$\alpha$-D-glucopyranosyl)-propan-2-one (27$\alpha$)**



As described for **27**$\beta$ with 4.000 g (11.68 mmol) **23**, 1.9 ml $SnCl_4$ (4.212 g, 16.170 mmol), 2.016 g (15.47 mmol) silylenolether of acetone in 200 ml dry $CH_3CN$ with 50 g 3Å molecular sieves resulted in a $\alpha$-D/$\beta$-D = 69/31 mixture of **27** (yield 2.71 g, 7.37 mmol, 63%).

$R_f$ ($SiO_2$ ; $Et_2O$/hexane 2:1) 0.20 ($Ce(SO_4)_2$).

IR (film): 3486br. *m*, 3088*m*, 3063*s*, 3030*s*, 2935*vs*, 2865*vs*, 1955*w*, 1876*w*, 1812*w*, 1713*vs*, 1661*w*, 1605*w*, 1586*w*, 1496*s*, 1453*vs*, 1362*vs*, 1313*s*, 1275*s*, 1206*s*, 1097br. *vs*, 1028*s*, 908*m*, 852*w*, 819*w*, 737*vs*, 698*vs*, 608*w*, 532*w*.

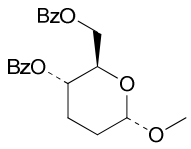
$^1$H-NMR (300 MHz, $CDCl_3$): 7.36-7.18 (*m*, 10 arom. H); 4.69-4.30 (*m*, 4 benz. H); 3.88-3.79, 3.72-3.61, 3.51-3.38 (3*m*, 5H, HC(1), $H_2$C(6), HC(4), HC(5)); 2.85 (*dd*, *J* = 15.7, 7.6, 1H of C(1)$H_2$CCO); 2.50 (*dd*, *J* = 15.7, 5.9, 1H of C(1)$H_2$CCO); 1.98-1.85, 1.81-1.62. 1.50-1.36 (3*m*, $H_2$C(2), $H_2$C(3)).

$^{13}$C-NMR: (75 MHz, $CDCl_3$): 207.2 (*s*, CO); 138.4, 138.2 (2*s*, arom. C); 128.3, 127.8, 127.71, 127.67, 127.57, 127.4 (6*d*, arom. C); 73.78, 72.0, 68.3 (3*d*, C(1), C(4), C(5)); 73.72, 70.6, 69.1 (3*t*, 2 O$CH_2$arom., C(6)); 47.1 (*t*, C(1)$CH_2$CO); 30.8 (*q*, Me); 26.5, 24.0 (2*t*, C(2), C(3)).

ESI-MS: 391.2 (100 $[M+Na]^+$).

HR-ESI-MS: calc. for $C_{23}H_{28}O_4Na_1$ ($[m+Na]^+$) 391.1885; found 391.1890.

**Methyl-4,6-di-*O*-benzoyl-2,3-dideoxy-$\alpha$-D-glucopyranoside (22)**




To a solution of **18** (3.25 g, 20.1 mmol) in 16 ml of pyridine was added at 0 °C BzCl (6.50 ml, 7.91 g, 56.3 mmol). After stirring for 6 h at rt, the pyridine was evaporated, the crude product was dissolved in 150 ml of EtOAc, washed with $H_2O$ (1 x 75 ml), aq. 1 M HCl (1 x 75 ml), aq. $CuSO_4$ (1 x 30 ml), aq. $NaHCO_{3\ sat.}$ (1 x 75 ml), $H_2O$ (1 x 75 ml), dried ($MgSO_4$) and evaporated. CC (200 g $SiO_2$, hexane/$Et_2O$ 2:1) yielded 6.16 g (16.6 mmol, 83%, $\alpha$-D/$\beta$-D = 14:1 (GC, $^1$H-NMR)) of **22** as a colourless solid.

$R_f$ ($SiO_2$ ; $Et_2O$/hexane 1:1) 0.26 ($UV_{254}$, $Ce(SO_4)_2$).

IR(film) 3606*vs* (br.), 2085*m*, 1787*m*, 1717*vs*, 1602*vs*, 1491*w*, 1451*s*, 1372*w*, 1356*w*, 1315*w*, 1268*s*, 1225*m*, 1212*m*, 1176*m*, 1112*s*, 1069*m*, 1053*s*, 1026*m*, 1012*m*, 999*m*, 972*w*, 951*w*, 804*w*, 778*w*, 709*vs*, 687*s*, 516*w*.

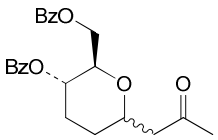
$^1$H-NMR (300 MHz, $CDCl_3$): 8.06-8.01 (*m*, 4 arom. H); 7.58-7.51 (*m*, 2 arom. H); 7.47-7.38 (*m*, 2 arom. H); 5.09 (*dt*, *J* = 10.1, 4.9, HC(4), $\alpha$-D); 4.78 (*t*, *J* = 2.2, HC(1), $\alpha$-D); 4.66-4.61 (*m*, 1H of $H_2C(6)$, $\beta$-D); 4.58 (*dd*, *J* = 11.8, 2.8, 1H of $H_2C(6)$, $\alpha$-D); 4.47-4.41 (m, 1H of $H_2C(6)$, $\beta$-D); 4.40 (*dd*, *J* = 11.8, 5.9, 1H of $H_2C(6)$, $\alpha$-D); 4.24 (*ddd*, *J* = 9.7, 5.9, 2.6, HC(5), $\alpha$-D); 3.51 (*s*, MeO, 0.21H, $\beta$-D); 3.42 (*s*, MeO, 3.00H, $\alpha$-D); 2.21-2.13, 2.08-1.90 (2*m*, $H_2C(2)$, $H_2C(3)$).

$^{13}$C-NMR: (75 MHz, $CDCl_3$): 166.3, 165.4 (2*s*, CO); 129.9, 129.8 (2*s*, arom. C); 133.1, 132.9, 129.6, 128.8, 128.3, 128.2 (6*d*, arom. C); 97.4 (*d*, C(1)); 68.9, 68.6 (2*d*, C(4), C(5)); 64.2 (*t*, C(6)); 54.6 (*q*, MeO); 28.7, 24.1 (2*t*, C(2), C(3)).

ESI-MS: 393.1 (100, $[M+Na]^+$).

HR-ESI-MS: calc. for $C_{21}H_{22}O_6Na_1$ ($[m+Na]^+$) 393.1314; found 393.1321.

**1-(4',6'-di-*O*-benzoyl-2',3'-dideoxy-$\alpha,\beta$-D-glucopyranosyl)-propan-2-one (26)**




As described for **27** from **22** (1.00 g, 2.70 mmol), silylenolether (422 mg, 3.24 mmol), $SnCl_4$ (0.44 ml, 984 mg, 3.78 mmol) in 35 ml $CH_3CN$ (except that to the solution was added finely ground and dried (60 h, 180° C) 3 Å molecular sieves (1.60 g)). CC (40 g $SiO_2$, $Et_2O$/hexane 3:1) yielded 469 mg (1.27 mmol, 47 %) of **26** as colourless oil (**26**$\alpha$-D/**26**$\beta$-D= 7:3 ($^1$H-NMR)).

$R_f$ ($SiO_2$ ; $Et_2O$/hexane 3:1) 0.20 ($UV_{254}$, $Ce(SO_4)_2$).

IR (film) 3426*w*, 3067*w*, 2958*m*, 2877*w*, 1719*vs*, 1602*m*, 1584*m*, 1492*w*, 1474*w*, 1451*s*, 1359*m*, 1318*s*, 1275*vs* (br.), 1176*m*, 1113*s*, 1070*s*, 1052*s*, 1026*s*, 987*m*, 938*m*, 898*m*, 806*w*, 711*vs*, 686*m*, 606*w*, 591*w*, 497*w*.

$^1$H-NMR (300 MHz, $CDCl_3$): 8.08-7.99, 7.58-7.53, 7.47-7.40 (3*m*, arom. H); 5.11 (*dt*-like, HC(4), 1.08H, $\alpha$-D); 5.01 (*dt*, *J* = 10.5, 4.8, HC(4), 0.33H, $\beta$-D); 4.61-4.48, 4.42-4.24 (2*m*, $H_2C(6)$, HC(1)); 3.98-3.86 (*m*, HC(5)); 2.87 (*dd*, *J* = 15.5, 7.9, 1.00H, 1H from C(1)C$H_2$CO, $\alpha$-D); 2.76 (*dd*, *J* = 15.9, 7.5, 0.34H, 1H from C(1)C$H_2$CO, $\beta$-D); 2.56 (*dd*, *J* = 15.5, 5.5, 1H of C(1)C$H_2$CO, $\alpha$-D); 2.48 (*dd*, *J* = 16.0, 5.1, 1H of C(1)C$H_2$CO, $\beta$-D); 2.40-2.38, 2.13-2.10, 2.04-1.93, 1.90-1.53 (4*m*, $H_2C(2)$, $H_2C(3)$).

$^{13}$C-NMR: (75 MHz, $CDCl_3$): 206.3 (*s*, $H_3CCO$), 166.2, 165.6 (2*s*, 2OCO); 133.1, 133.0, 129.7, 129.6, 128.32, 128.29 (6*d*, arom. C); 77.4, 74.1, 72.5, 69.0, 68.0, 67.9 (6*d*, C(1), C(4), C(5)); 64.3, 62.7 (2*t*, C(6)); 49.0 (*t*, C(1)$CH_2CO$, $\beta$ -D);, 47.9 (*t*, C(1)$CH_2CO$, $\alpha$-D); 30.34 (*q*, Me); 30.33, 29.1, 26.3, 24.3 (4*t*, C(2), C(3)).

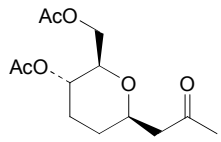
ESI-MS: 419 (100, $[M+Na]^+$).

HR-ESI-MS: calc. for $C_{23}H_{24}O_6Na_1$ ($[m+Na]^+$) 419.1471; found 419.1477.

**1-(4',6'-di-*O*-acetyl-2',3'-dideoxy-$\beta$-D-glucopyranosyl)-propan-2-one (25$\beta$)**

**1-(4',6'-di-*O*-acetyl-2',3'-dideoxy-$\alpha$-D-glucopyranosyl)-propan-2-one (25$\alpha$)**

As described for **27**, with 100 mg (0.406 mmol) of **17**. CC (20 g $SiO_2$, $Et_2O$: hexane 3:1) (to increase the solubility of the crude material, several drops of $CH_2Cl_2$ were added) yielded 25 mg (91.8 µmol, 23%) of the $\alpha$-D-anomer of **25** and 24 mg (88.1 mmol, 22%) of the $\beta$-D-anomer of **25**.




$R_f$ ($SiO_2$ ; $Et_2O$) 0.58 ($UV_{254}$, $Ce(SO_4)_2$).

IR (film): 3467 br. *m*, 2953*m*, 2871*m*, 1740*vs*, 1439*m*, 1372*s*, 1243*vs*, 1165*m*, 1110*m*, 1041*s*, 995*m*, 910*w*, 871*w*, 650*w*, 605*w*, 573*m*, 539*w*.

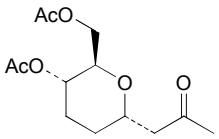
$^{1}$H-NMR (600 MHz, $CDCl_3$): 4.65 (*dt*, *J* = 10.2, 4.8, HC(4)); 4.20 (*dd*, *J* = 12.0, 5.7, 1H of $H_2C(6)$); 4.09 (*dd*, *J* = 12.0, 2.3, 1H of $H_2C(6)$); 3.86-3.81 (*m*, HC(1)); 3.36 (*ddd*, *J* = 9.9, 5.7, 2.3, HC(5)); 2.75 (*dd*, *J* = 16.1, 7.2, 1H of C(1)C$H_2$CO); 2.46 (*dd*, *J* = 16.1, 5.3, 1H of C(1)C$H_2$CO); 2.24-2.19 (*m*, $HC(3)_{eq}$); 2.06, 2.04 (2*s*, 2Ac); 1.85-1.75 (*m*, $HC(2)_{eq}$); 1.56-1.49 (*m*, $HC(3)_{ax}$) ; 1.48-1.42 (*m*, $HC(2)_{ax}$).

$^{13}$C-NMR: (125 MHz, $CDCl_3$): 206.7 (*s*, CO); 170.9, 170.1 (2*s*, 2 CO from Ac); 77.4, 74.0, 67.9 (3*d*, C(1), C(4), C(5)); 63.5 (*t*, C(6)); 49.1 (*t*, C(1)$C$H$_2$CO); 31.0 (*q*, H$_3C$COCH$_2$); 30.3, 29.0 (2*t*, C(2), C(3)); 22.0, 20.9 (2*q*, 2$CH_3$ from Ac).

CI-MS: 290.2 (100, $[M+NH_4]^+$), 273.2 (18, $[M+H]^+$).

ESI-MS: 295.2 (100, $[M+Na]^+$).

HR-ESI-MS: calc. for $C_{13}H_{20}O_3Na_1$ ($[m+Na]^+$) 295.1158; found 295.1156.




$R_f$ ($SiO_2$ ; $Et_2O$) 0.50 ($UV_{254}$, $Ce(SO_4)_2$).

IR (film): 3459 br. *vs*, 2083*w*, 1642*s*, 1441*w*, 1370*w*, 1255*m*, 1137*w*, 1042*w*, 968*w*, 733*m*, 607*m*.

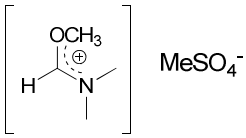
$^{1}$H-NMR (600 MHz, $CDCl_3$): 4.79-4.71 (*m*, HC(4)); 4.34 (*dd*, *J* = 11.8, 6.9, 1H of $H_2C(6)$); 4.29-4.22 (*m*, HC(1)); 4.11 (*dd*, *J* = 11.8, 4.5, 1H of $H_2C(6)$); 3.95-3.91 (*m*, HC(5)); 2.81 (*dd*, *J* = 15.7, 7.9, 1H of C(1)C$H_2$CO); 2.50 (*dd*, *J* = 15.7, 5.5, 1H of C(1)C$H_2$CO); 2.08, 2.07 (2*s*, 2Ac); 2.00-1.88 (*m*, 1HC(3)); 1.83-1.77 (*m*, 1HC(3)); 1.77-1.71 (*m*, 1HC(2)); 1.67-1.60 (*m*, 1HC(2)).

$^{13}$C-NMR: (125 MHz, $CDCl_3$): 206.3 (*s*, CO); 170.8, 170.3 (2*s*, 2 CO from Ac); 72.4, 67.8, 67.2 (3*d*, C(1), C(4), C(5)); 62.0 (*t*, C(6)); 47.7 (*t*, C(1)*C*$H_2$CO); 30.5 (*q*, $H_3CCOCH_2$); 26.2, 24.2 (2*t*, C(2), C(3)); 21.2, 20.8 (2*q*, 2$CH_3$ from Ac).

ESI-MS: 295.1 (100, $[M+Na]^+$).

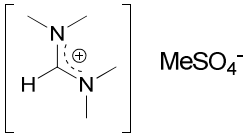
HR-ESI-MS: calc. for $C_{13}H_{20}O_3Na_1$ ($[m+Na]^+$) 295.1158; found 295.1156.

***N*,*N*-dimethyl-formimidiumacid-methylester-methylsulfate (30)**




To 10 g (0.137 mol) dry DMF was added at rt 13 ml (17.2 g, 0.136 mol) $Me_2SO_4$ under Ar. The reaction was stirred for 30 min at rt and 3 h at 60° C. The slightly yellow solution was cooled to rt and the crude product **30** (27.2 g, 0.136 mol) was used without purification in the preparation of **31**.

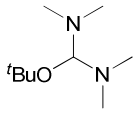
***N*,*N*,*N'*,*N'*-tetramethyl-formamidinium-methylsulfate (31)**




27.2 g (0.136 mol) of crude **30** was added dropwise over 45 min at rt to a saturated solution of $Me_2NH$ in 100 ml dry benzene (prepared by bubbling 26.2 g (0.584 mol) $Me_2NH$ through a stirred solution of 100 ml benzene at 0° C and carefully heating to rt). The reaction was heated to 80° C

for 1 h, cooled to rt and the lower, golden layer was separated, extracted with dry $Et_2O$ (2 x 50 ml) and dried under vacuum ($p < 10^{-1}$ mbar) resulting in 22.0 g (0.104 mol, 76% from DMF) of **31** as a colourless low melting solid.

***Bredereck*'s reagent (29)**




15.5 g (73.0 mmol) of **31** was added under Ar as solid during 1 h to a solution of 9.20 g (82.0 mmol) K$^t$BuO in 150 ml dry $Et_2O$. The reaction was stirred for 1.5 h at rt. The yellow suspension was filtered and the solvent evaporated ($t$ = 40° C, $p$ = 500 mbar). Vacuum-distillation of the yellow oil ($p$ = 11 mbar, $t$ = 60 °C) resulted in 7.30 g (41.9 mmol, 57%) of *Bredereck*'s reagent **29** as a colourless oil.

$^1$H-NMR ($C_6D_6$, 300 MHz): 4.08 (*s*, CH); 2.32 (*s*, 12H of $3NCH_3$); 1.18 (*s*, $OC(CH_3)_3$).

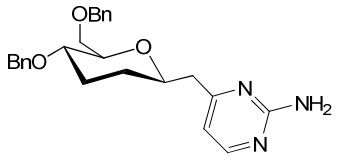
$^{13}$C-NMR ($C_6D_6$, 75 Mhz): 100.7 (*d*, CH), 72.0 (*s*, O*C*(CH3)3); 41.2 (*q*, N*C*$H_3$); 29.0 (*q*, $OC(CH_3)_3$).

**2-amino-4-[(4',6'-di-*O*-benzyl-2',3'-dideoxy-$\beta$-D-glucopyranosyl)methyl]-pyrimidine (34$\beta$)**

**2-amino-4-methyl-5-(4',6'-di-*O*-benzyl-2',3'-dideoxy-$\beta$-D-glucopyranosyl)-pyrimidine (35$\beta$)**

To a solution of **27$\beta$** (1.00 g, 2.71 mmol) in 12 ml dry toluene (*Acros*, over molecular sieves) was added *Bredereck*'s reagent (0.84 ml, 705 mg, 3.93 mmol). After stirring for 22 h at 70 °C, cooling to rt, adding 0.84 ml (705 mg, 3.93 mmol) *Bredereck*'s reagent and stirring for 90 min at 110 °C, the crude enaminoketone was cooled to rt, evaporated and dried for 12 h under vacuum ($p < 10^{-1}$

mbar). The enaminoketone was dissolved in 17 ml EtOH and added to a freshly prepared 1 M solution of NaOEt in EtOH (11 ml) and 1.16 g (5.36 mmol) guanidiniumsulfate, which had been stirred for 30 min at rt. The reaction was stirred for 6 h at 78 °C, evaporated, dissolved in $CH_2Cl_2$ (50 ml), washed with $H_2O$ (30 ml), dried ($Na_2SO_4$) and evaporated. CC (40 g $Al_2O_3$ Brockmann activity III, EtOAc/hexane 2:1) yielded 0.289 g (0.552 mmol, 20%) **34*β*** as a slightly yellow highly viscous oil, 0.428 g (0.817 mmol, 30%) **34*β*/35*β*** 1.5:1 ($^1$H-NMR) and 0.188 g (0.359 mmol, 13%) **35*β*** as a colourless solid.




$R_f$ ($Al_2O_3$; EtOAc/hexane 2:1) 0.25 ($UV_{254}$, $Ce(SO_4)_2$).

IR (film): 3337*vs* (br.), 3063*m*, 3030*m*, 2935*m*, 2863*m*, 2079*w*, 1955*w*, 1812*w*, 1631*vs*, 1578*vs*, 1495*m*, 1459*s*, 1369*m*, 1341*m*, 1315*w*, 1265*w*, 1205*m*, 1100*s*, 1027*m*, 1001*w*, 907*w*, 880*w*, 800*w*, 781*w*, 736*s*, 698*s*, 646*w*, 607*w*.

$^1$H-NMR ($CDCl_3$, 600 MHz) 8.11 (*d*, 1H, *J* = 5.1 Hz, NC*H*$_{arom.}$), 7.33-7.21 (*m*, 10H, arom. H), 6.64 (*d*, *J* = 5.1 Hz, NCHC*H*$_{arom.}$), 5.29 (br. *s*, $NH_2$), 4.63-4.40 (*m*, 4H, benz. H), 3.76-3.71 (*m*, 1H of $H_2C(6')$, HC(1')), 3.66 (*dd*, *J* = 10.8, 4.8 Hz, 1H of $H_2C(6')$), 3.44-3.39 (*m*, *H*C(4'), *H*C(5')), 2.88 (*dd*, *J* = 14.0, 7.6 Hz, 1H of HC(1')C*H*$_2$-arom.), 2.68 (*dd*, *J* = 14.0, 5.1 Hz, 1H of HC(1')C*H*$_2$-arom.), 2.29-2.26 (*m*, *H*C(3')$_{eq.}$), 1.79-1.76 (*m*, *H*C(2')$_{eq.}$), 1.50-1.40 (*m*, *H*C(3')$_{ax.}$, *H*C(2')$_{ax.}$).

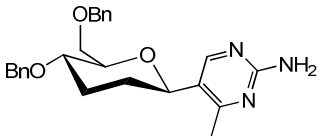
$^{13}$C-NMR ($CDCl_3$, 150 MHz): 169.1 (*s*, C(1')$CH_2C_{arom}$); 162.1 (*s*, $H_2NC_{arom}$); 157.3 (*d*, N*C*H$_{arom}$); 138.5, 138.4 (2*s*, 2 $OCH_2C_{arom}$); 128.4, 128.3, 127.7, 127.6, 127.5 (5*d*, 5 $C_{arom}$); 111.9 (*d*,

NCH*C*H$_{arom}$); 80.7 (*d*, *C*(5')); 76.3 (*d*, *C*(1')); 73.3 (*t*, 1 O*C*H$_2$-arom); 73.1 (*d*, *C*(4')); 71.0 (*t*, 1 O*C*H$_2$-arom); 69.8 (*t*, *C*(6')); 43.8 (*t*, C(1')*C*H$_2$-arom); 30.6 (*t*, *C*(2')); 29.2 (*t*, *C*(3')).

ESI-MS: 458.1 (5, [m+K]$^+$), 442.2 (6, [m+Na]$^+$), 420.3 (100, [m+H]$^+$).

HR-ESI-MS: calc. for $C_{25}H_{30}N_3O_3$ ([m+H]$^+$) 420.2287; found 420.2287.

Anal. calc. for $C_{25}H_{29}N_3O_3$ (419.52) C 71.57 H 6.97; found C 71.62 H 6.94.




$R_f$ ($Al_2O_3$; EtOAc/hexane 2:1) 0.22 ($UV_{254}$, $Ce(SO_4)_2$).

IR (KBr): 3408*vs* (br.), 2941*m*, 2864*w*, 2084*w* (br.), 1635*vs*, 1469*m*, 1367*w*, 1313*w*, 1216*w*, 1216*w*, 1074*m*, 1027*w*, 1000*w*, 909*w*, 798*m*, 735*m*, 697*s*.

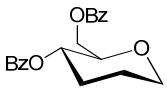
$^1$H-NMR ($CDCl_3$, 300 MHz): 8.24 (*s*, NC*H*$_{arom}$); 7.32-7.28 (*m*, 10 arom. H); 5.05 (br. *s*, $NH_2$); 4.65-4.39 (*m*, 5H, benz. H, HC(1')); 3.82-3.70 (*m*, 2H); 3.60 (*ddd*, *J* = 9.1, 4.9, 2.0, 1H); 3.54-3.46 (*m*, 1H); 2.40 (*s*, $H_3CC_{arom}$); 1.97-1.91, 1.80-1.42 (2*m*, $H_2C(2')$, $H_2C(3')$).

$^{13}$C-NMR ($CDCl_3$, 75 MHz): 166.1 (*s*); 161.5 (*s*), 155.5 (*d*), 138.4, 138.2 (2*s*, 2 OCH$_2$*C*$_{arom}$); 128.3, 128.2, 127.6, 127.5, 127.4 (5*d*, 5 C$_{arom}$); 123.0 (*s*); 81.2, 74.6, 72.8 (3*d*); 73.3, 71.0, 69.8 (3*t*); 30.5, 29.6 (2*t*, C(2), C(3)); 21.5 (*q*, H$_3$*C*C$_{arom}$).

ESI-MS: 442.2 (100, [m+Na]$^+$), 420.2 (34, [m+H]$^+$).

Anal. calc. for $C_{25}H_{29}N_3O_3$ (419.52) C 71.57 H 6.97 N 10.02; found C 71.44 H 6.87 N 9.87.

**(2*R*, 3*S*)-2-(benzoylmethoxy)-3-(benzoylhydroxy)tetrahydropyrane (86)**




To a solution of 220 mg (0.650 mmol) unsaturated pyranoside **56** in 3 ml MeOH and 0.03 ml AcOH at rt was added 5 mg of 10% Pd(C). The solution was evacuated and flushed with $H_2$ three times, stirred for 16 h under $H_2$, and filtered over Celite. The solvent was evaporated under reduced pressure and the crude material was dissolved in 150 ml EtOAc, extracted with $NaHCO_3$ $_{sat.}$ (2 x 70 ml), NaCl $_{sat.}$ (1 x 50 ml), the combined aqueous phases were extracted with EtOAc (40 ml) and the combined organic phases were dried over $Na_2SO_4$ and evaporated under reduced pressure, resulting in 218 mg (0.640 mmol) of **86** as colourless highly viscous oil.

$R_f$ ($SiO_2$; EtOAc) 0.81 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, $CDCl_3$): 8.05-8.01 (*m*, 4 arom. H); 7.57-7.49 (*m*, 2 arom. H); 7.43-7.36 (*m*, 4 arom. H); 5.11-5.02 (*m*, 1H of $H_2C(1)$, HC(4), HC(5)); 4.61 (*dd*, *J* = 11.9, 2.6, 1H of $H_2C(6)$); 4.38 (*dd*, *J* = 11.9, 5.7, 1H of $H_2C(6)$); 4.05-4.00 (*m*, 1H of $H_2C(1)$, HC(4), HC(5)); 3.81 (*ddd*, *J* = 8.4, 5.6, 2.6, 1H of $H_2C(1)$, HC(4), HC(5)); 3.48 (*dt*, *J* = 11.7, 2.6, 1H of $H_2C(1)$, HC(4), HC(5)); 2.43-2.38 (*m*, 1H of $H_2C(2)$, $H_2C(3)$); 1.94-1.60 (*m*, 3H of $H_2C(2)$, $H_2C(3)$).

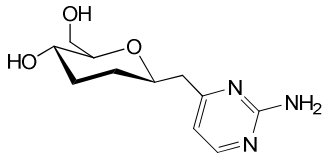
$^{13}$C-NMR (75 MHz, $CDCl_3$): 171.0 (*s*, CO); 166.4, 165.5 (2*s*, arom. C); 133.1, 132.9, 129.93, 129.86, 128.4, 128.3 (6*d*, arom. C); 77.7, 69.2 (2*d*, C(4), C(5)); 67.9, 64.4 (2*t*, C(1), C(6)); 29.4, 24.9 (2*t*, C(2), C(3)).

CI-MS: 218.0 (24); 205.0 (10), 104.9 (100), 76.9 (21), 27.9 (5).

ESI-MS: 368.1 (100, $[m+Na]^+$).

HR-ESI-MS: calc. for $C_{20}H_{20}O_5Na$ ($[m+Na]^+$) 368.1208; found 368.1208.

**2-amino-4-[(2',3'-dideoxy-*β*-D-glucopyranosyl)methyl]-pyrimidine (10)**




To a solution of 492 mg (1.17 mmol) **34*β*** in 100 ml $CH_2Cl_2$ under $N_2$ at 0 °C was added 4.7 ml of a 1 M solution of $BBr_3$ in $CH_2Cl_2$ (4.70 mmol). After stirring for 3 min at 0 °C, the reaction was quenched by slowly adding 0.996 g (9.397 mmol) $Na_2CO_3$ in 30 ml $H_2O$, followed by 40 ml $H_2O$. The pH was adjusted to 9 with 2 N $NaOH_{aq}$, the aqueous phase was separated and the organic phase was extracted with $H_2O$ (30 ml). The combined aqueous phases were washed with $CH_2Cl_2$ (30 ml) and evaporated under reduced pressure. CC (5 g $SiO_2$, MeOH/$CH_2Cl_2$ 1:4) yielded 210 mg (0.878 mmol, 75%) of **10** as colourless solid.

$R_f$ ($SiO_2$; $CH_2Cl_2$/MeOH 4:1) 0.27 ($UV_{254}$, $Ce(SO_4)_2$).

UV($H_2O$) 292 (2325).

IR (film) 3345*vs* (br.), 2937*s*, 2864*s*, 1627*vs*, 1583*vs*, 1567*vs*, 1468*vs*, 1383*m*, 1373*m*, 1343*m*, 1216*m*, 1073*s*, 988*w*, 799*w*.

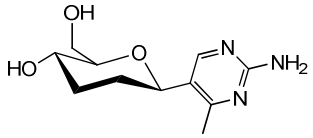
$^{1}$H-NMR (500 MHz, $D_2O$): 8.11 (*d*, *J* = 5.1, RNC*H*$_{arom}$); 6.65 (*d*, *J* = 5.1, NCC*H*$_{arom}$); 3.77 (*dd*, *J* = 11.5, 2.4, 1HC(6')); 3.76-3.71 (*m*, HC(1')); 3.74 (*dd*, *J* = 11.1, 5.2, 1HC(6')); 3.42-3.35 (*m*, HC(4')); 3.13 (*ddd*, *J* = 8.7, 5.5, 2.5, HC(5')); 2.80 (*dd*, *J* = 14.0, 7.4 , 1H of HC(1')C*H*$_2$-arom.); 2.66 (*dd*, *J* =13.9, 5.2, 1H of HC(1')C*H*$_2$-arom.); 2.10-2.04 (*m*, C(3')H$_{eq}$); 1.76-1.71 (*m*, C(2')H$_{eq}$); 1.52-1.34 (*m*, C(3')H$_{ax}$, C(2')H$_{ax}$).

$^{13}$C-NMR (125 MHz, $D_2O$): 170.8 (*s*, $CH_2C_{arom}$); 164.5 (*s*, $H_2NC_{arom}$); 158.9 (*d*, $NCH_{arom}$); 112.2 (*d*, NCH*C*H$_{arom}$); 84.1 (*d*, C(5')); 77.5 (*d*, C(1')); 67.4 (*d*, C(4')); 63.5 (*t*, C(6')); 44.8 (*t*, C(1')*C*H$_2$-arom); 33.7 (*t*, C(3')); 32.1 (*t*, C(2')).

ESI-MS: 262 (100, $[m+Na]^+$), 240 (45, $[m+H]^+$).

HR-ESI-MS: calc. for $C_{11}H_{18}N_3O_3$ ($[m+H]^+$) 240.1348; found 240.1347.

**2-amino-4-methyl-5-(2',3'-dideoxy-$\beta$-D-glucopyranosyl)-pyrimidine (36)**




To a solution of 114 mg (0.218 mmol) **35** in 30 ml dry $CH_2Cl_2$ under $N_2$ was added at 0 °C 1.1 ml of a 1 M $BBr_3$ (1.1 mmol) solution in $CH_2Cl_2$. The reaction was stirred for 3 min at 0 °C, quenched by a dropwise addition of 17 ml $H_2O$ at 0 °C and the pH of the aq. phase was adjusted to 11 with aq. $NaHCO_{3\ sat.}$ and aq. 2 N NaOH, the aq. phase was evaporated, the remaining solid was dissolved in 20 ml MeOH, filtered and evaporated. CC (6 g $Al_2O_3$; $CH_2Cl_2$/MeOH 5:1) resulted in 41 mg (0.171 mmol, 79 %) **36** as a colourless solid.

$R_f$ ($SiO_2$ ; $CH_2Cl_2$/MeOH 5:1) 0.19 ($UV_{254}$, $Ce(SO_4)_2$).

$R_f$ ($Al_2O_3$ ; $CH_2Cl_2$/MeOH 5:1) 0.41 ($UV_{254}$, $Ce(SO_4)_2$).

IR(film): 3345*vs* (br.), 2937*s*, 2864*s*, 1627*vs*, 1583*vs*, 1567*vs*, 1468*vs*, 1383*m*, 1373*m*, 1343*m*, 1216*m*, 1073*s*, 988*w*, 799*w*.

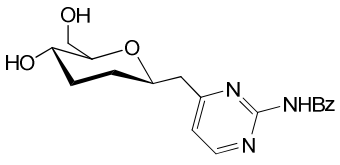
$^{1}$H-NMR (600 MHz, $D_2O$): 8.21 (*s*, NC*H*$_{arom.}$), 4.56 (*dd*, *J* = 11.4, 1.9, HC(1')); 3.88 (*dd*, *J* = 12.3, 2.3, 1H of $H_2C$(6')); 3.72 (*dd*, *J* = 12.3, 6.0, 1H of $H_2C$(6')); 3.60 (*dt*, *J* = 9.7, 4.8, HC(4')); 3.50 (*ddd*, *J* = 9.4, 6.0, 2.3, HC(5')); 2.26-2.21 (*m*, C(3')$H_{eq}$)); 1.96-1.92 (*m*, C(2')$H_{eq}$)); 1.88-1.81 (*m*, C(2')$H_{ax}$)); 1.71-1.65 (*m*, C(3')$H_{ax}$)).

$^{13}$C-NMR (150 MHz, $D_2O$): 168.4 (*s*, $H_2NC_{arom}$); 161.4 (*s*, $H_3CC_{arom}$); 155.7 (*d*, $HC_{arom}$); 122.1 (*s*, C(1')$C_{arom}$); 82.6 (*d*, C(5')); 74.1 (*d*, C(1')); 65.6 (*d*, C(4')); 61.4 (*t*, C(6')); 31.7 (*t*, C(3')); 29.2 (*t*, C(2')); 20.5 (*q*, $H_3C$).

ESI-MS: 262.1 (100, $[M+Na]^+$), 240 .1(21, $[M+H]^+$).

HR-ESI-MS: calc. for $C_{11}H_{18}N_3O_3$ ($[m+H]^+$) 240.1348; found 240.1348.

**2-benzoylamino-4-[(2',3'-dideoxy-β-D-glucopyranosyl)methyl]-pyrimidine (73)**




To a suspension of 281 mg (1.173 mmol) **10** in dry pyridine at 0 °C was added dropwise 0.74 ml (636 mg, 5.864 mmol) $Me_3SiCl$. After stirring for 30 min at 0 °C, 0.68 ml (824 mg, 5.864 mmol) benzoyl chloride was added dropwise to the solution at 0° C. The reaction was stirred for 2 h at rt, cooled to 0 °C and 2.4 ml $H_2O$ was added, stirred for 15 min followed by the addition of 2.6 ml

$NH_{3\ conc}$ (25% in $H_2O$, 13.4 M). After stirring for 30 min, the pyridine was evaporated, the product was dissolved in 30 ml $H_2O$ and washed with 10 ml $Et_2O$. CC (12 g $SiO_2$; $CH_2Cl_2$/MeOH 10:1 to $CH_2Cl_2$/MeOH 8:1) resulted in 321 mg (0.935 mmol, 80 %) **73** as a colourless foam.

M.p. 65 °C.

$R_f$ ($SiO_2$ ; $CH_2Cl_2$/MeOH 10:1) 0.35 ($UV_{254}$, $Ce(SO_4)_2$).

IR (KBr): 3416*vs* (br.), 2937*w*, 2865*w*, 2639*w*, 1690*s*, 1597*vs*, 1557*m*, 1527*s*, 1440*vs*, 1404*m*, 1316*w*, 1267*m*, 1073*m*, 1027*w*, 796*w*, 712*m*, 650*w*, 624*w*, 578*w*.

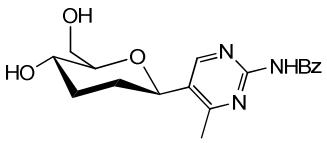
$^1$H-NMR (300 MHz, d$^4$-MeOD): 8.58 (*d*, *J* = 5.1, NC*H*$_{arom}$); 8.08-7.97 (*m*, 2 COCC*H*$_{arom}$); 7.68-7.42 (*m*, 3 arom. H); 7.24 (*d*, *J* = 5.1, RNCHC*H*$_{arom}$); 3.90-3.82 (*m*, HC(1')); 3.77 (*dd*, *J* = 11.7, 2.7, 1HC(6')); 3.65 (*dd*, *J* = 11.7, 5.5, 1HC(6')); 3.46-3.36 (*m*, HC(4')); 3.15 (*ddd*, *J* = 8.6, 5.5, 2.7, HC(5')); 2.97 (*dd*, *J* = 13.8, 7.4, 1H of HC(1')C*H*$_2$-arom.); 2.91 (*dd*, *J* = 13.3, 2.9, 1H of HC(1')C*H*$_2$-arom.); 2.17-2.03, 1.82-1.74, 1.58-1.46 (3*m*, $H_2C(2')$, $H_2C(3')$).

$^{13}$C-NMR (75 MHz, d$^4$-MeOD): 171.5, 168.6, 159.1 (3*s*, *C*O, $CH_2C_{arom}$, NH*C*$_{arom}$), 135.7 *(s*, (CO)*C*$_{arom}$); 133.7, 130.8, 129.6 (3*d*, arom. C); 118.9 (*d*, NCH*C*H$_{arom}$); 84.1 (*d*, C(5')); 77.4 (*d*, C(1')); 67.4 (*d*, C(4')); 63.5 (*t*, C(6')); 44.7 (*t*, C(1')*C*$H_2$-arom.); 33.7, 32.1 (2*t*, C(3'), C(2')).

ESI-MS: 366 (100, $[m+Na]^+$), 344 (29, $[m+H]^+$).

HR-ESI-MS: calc. for $C_{18}H_{21}N_3O_4Na$ ($[m+Na]^+$) 366.1430; found 366.1428.

**2-aminobenzoyl-4-methyl-5-(2',3'-dideoxy-β-D-glucopyranosyl)-pyrimidine (74)**




To a suspension of 684 mg (2.86 mmol) **36** in 29 ml dry pyridine was added 1.80 ml (1.55 g, 14.23 mmol) $Me_3SiCl$ dropwise over 5 min. The reaction was stirred for 30 min at 0 °C, 1.66 ml (2.01 g, 14.31 mmol) of BzCl was added dropwise at 0° C and the reaction was stirred for 2 h at 24° C and cooled to 0° C. 5.8 ml ice cold water, followed by 6.3 ml (25%, 13.4M) $NH_{3aq.}$, was added and the reaction was stirred for 30 min at 0° C and evaporated under reduced pressure, dissolved in 100 ml $H_2O$ and extracted with $Et_2O$ (3 x 25 ml). The aqueous phase was evaporated and the resulting colourless solid was suspended under ultrasonic in MeOH (50 ml). The suspension was filtered and the colourless solid washed with MeOH (2 x 10 ml) and the MeOH evaporated. CC (12 g $SiO_2$, $CH_2Cl_2$/MeOH 10:1) resulted in 582 mg (1.70 mmol, 59%) **74** as a slightly yellow foam.

$R_f$ ($SiO_2$ ; $CH_2Cl_2$/MeOH 10:1) 0.25 ($UV_{254}$, $Ce(SO_4)_2$).

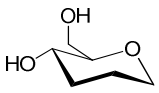
$^1$H-NMR (300 MHz, d$^6$-DMSO): 10.89 (br. *s*., N*H*CO); 8.62 (*s*, $HC_{arom}$); 7.98-7.94 (*m*, arom. H); 7.64-7.43 (*m*, arom. H); 4.55 (*m*, HC(1')); 3.78-3.73, 3.57-3.50, 3.49-3.36, 3.29-3.24 (4*m*, each 1H of HC(4'), HC(5'), $H_2C(6')$); 2.08-2.05, 1-95-1.92 (2*m*, each 1H of $H_2C(2')$, $H_2C(3')$); 1.71-1.53 (*m*, 2H of $H_2C(2')$, $H_2C(3')$).

$^{13}$C-NMR (75 MHz, d$^6$-DMSO): 167.6, 165.8 (2*s*, CO, HN$C_{arom}$); 156.9 (*s*, $CH_3C_{arom}$); 155.3 (*d*, HC(6)); 134.3 (*s*, CO$C_{arom}$); 134.3, 132.7, 132.0 (3*d*, $C_{arom}$); 83.8, 73.5, 65.0 (3*d*, C(5'), C(1'), C(4')); 61.6 (*t*, C(6')); 32.8, 30.5 (2*t*, C(2'), C(3')); 21.4 (*q*, $H_3C$).

ESI-MS: 366 (100, $[m+Na]^+$), 344 (8, $[m+H]^+$).

HR-ESI-MS: calc. for $C_{18}H_{21}N_3O_4Na$ ($[m+Na]^+$) 366.1430; found 366.1426.

**(2*R*, 3*S*)-2-(hydroxymethoxy)-3-hydroxy-tetrahydropyrane (87)**



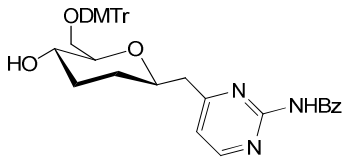

A solution of 240 mg (0.705 mmol) **86** was stirred for 24 h in a saturated $NH_3$ solution in $CH_3OH$. The solvent was evaporated and the crude mixture was dissolved in $H_2O$ (40 ml), extracted with $CH_2Cl_2$ (3 x 20 ml), and the combined organic phases were extracted with $H_2O$ (30 ml). The combined aqueous phases were lyophilised, resulting in 73 mg (0.552 mmol, 78 %) of **87** as colourless highly viscous oil.

$R_f$ ($SiO_2$ ; EtOAc) 0.13 ($Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, d$^4$-MeOD): 3.88-3.77 (*m*, 2H); 3.56 (*dd*, *J* = 11.6, 6.2, 1H); 3.36-3.26 (*m*, 2H); 3.04 (*ddd*, *J* = 9.1, 6.2, 2.6, 1H); 2.06-2.00 (*m*, 1H of $H_2C(2)$, $H_2C(3)$); 1.65-1.57 (*m*, 2H of $H_2C(2)$, $H_2C(3)$); 1.45-1.32 (*m*, 1H of $H_2C(2)$, $H_2C(3)$).

$^{13}$C-NMR (75 MHz, d$^4$-MeOD): 84.3 (*d*); 68.6 (*t*); 67.6 (*d*); 63.5 (*t*); 33.7 (*t*); 26.7 (*t*).

**2-benzoylamino-4-{[2',3'-dideoxy-6'-*O*-({4,4'-dimethoxytriphenyl}methyl)-$\beta$-D-glucopyranosyl]methyl}-pyrimidine (75)**




265 mg (0.772 mmol) **73**, dried for 15 h under high vacuum ($p < 10^{-1}$ mbar) was coevaporated with 1 ml pyridine, 317 mg (0.927 mmol) $Bu_4NClO_4$ and 314 mg (0.927 mmol) DMTrCl were added and dried for 1.5 h under high vacuum. 4 ml pyridine was added and the reaction was stirred for 2 h, 15 min at rt. 60 ml $CH_2Cl_2$ were added and the reaction was extracted with aq. $NaHCO_{3\ sat.}$ (2 x 30 ml), aq. $NaCl_{\ sat.}$ (1 x 30 ml), dried ($Na_2SO_4$). CC (19 g $SiO_2$; EtOAc) (for this application, a few drops of $CH_2Cl_2$ were added due to solubility reasons) resulted in 422 mg (0.654 mmol, 85 %) **75** as a colourless foam.

M.P.: 150 – 155 °C.

$R_f$ ($SiO_2$; EtOAc) 0.28 ($UV_{254}$, $Ce(SO_4)_2$).

IR (KBr): 3441*vs* (br.), 3061*w*, 2963*m*, 2936*m*, 2876*m*, 2043*w*, 1691*m*, 1604*s*, 1510*s*, 1440*vs*, 1403*m*, 1383*w*, 1301*m*, 1250*s*, 1177*m*, 1094*vs* (br.), 1032*s*, 999*w*, 931*w*, 884*w*, 830*w*, 708*m*, 623*m*, 597*w*, 583*w*, 515*w*.

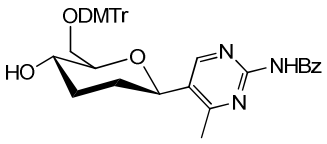
$^1$H-NMR (300 MHz, $CD_2Cl_2$): 8.68 (br. *s*, N*H*); 8.52 (*d*, *J* = 5.1, RNC$H_{arom}$); 7.73 (*dd*, *J* = 7.2, 1.4, 2 arom. H); 7.60 (*tt*, *J* = 6.4, 2.2, 1 arom. H); 7.51-7.40 (*m*, 4 arom. H); 7.32-7.18 (*m*, 7 arom. H); 7.07 (*d*, *J* = 5.1, RNCHC$H_{arom}$); 6.79 (*dd*, *J* = 9.0, 1.2, 4 arom. H); 3.84-3.75 (*m*, 2H of HC(1'), HC(4'), HC(5'), $H_2C(6')$); 3.73 (*s*, MeO); 3.72 (*s*, MeO); 3.50-3-38, 3.36-3.28, 3,28-3.21 (3*m*, each 1H, 3H from HC(1'), HC(4'), HC(5'), $H_2C(6')$); 2.94 (*d*, *J* = 6.5, HC(1')C$H_2$-arom.); 2.12-2.01, 1.84-1.76, 1.55-1.49 (3*m*, $H_2C(2')$, $H_2C(3')$).

$^{13}$C-NMR (75 MHz, $CD_2Cl_2$): 170.0, 165.2, 158.2 (3*s*, *C*O, $CH_2$***C***$_{arom}$, NH*C*$_{arom}$), 158.9, 157.7 (2*s*, arom. C); 158.2 (*d*, $NCH_{arom}$); 136.1, 136.0, 134.6 (3*s*, (CO)*C*$_{arom}$, 2 arom. C); 130.3, 129.0, 128.3, 128.2, 127.7, 127.1, 113.4 (7*d*, arom. C); 117.4 (*d*, NCH*C*H$_{arom}$); 86.7 (*s*, *C*OC(6')); 80.7 (*d*, C(5')); 76.4 (*d*, C(1')); 68.6 (*d*, C(4')); 65.4 (*t*, C(6')); 55.4 (*q*, OMe); 44.0 (*t*, C(1')*C*$H_2$-arom.); 32.5, 30.9 (2*t*, C(2'), C(3')).

ESI-MS: 668.3 (100, $[m+Na]^+$).

HR-ESI-MS: calc. for $C_{39}H_{39}N_3O_6Na$ ($[m+Na]^+$ 668.2737; found 668.2745.

**2-aminobenzoyl-4-methyl-5-{2',3'-dideoxy-6'-*O*-([4,4'-dimethoxytriphenyl]methyl)-*β*-D-glucopyranosyl}-pyrimidine (76)**




545 mg (1.59 mmol) **74**, 647 mg (1.91 mmol) DMTrCl, and 653 mg (1.91 mmol) $(Bu_4N)ClO_4$ were dried for 2 h under high vacuum ($p < 10^{-1}$ mbar) and dissolved in 8 ml pyridine, resulting in a red, clear solution. The reaction was stirred for 2 h 30 min at rt and evaporated under reduced pressure ($t$ = 30° C) to a volume of 1 ml. $CH_2Cl_2$ (60 ml) was added and the reaction was extracted with $NaHCO_{3\ sat.}$ (2 x 30 ml), $NaCl_{sat.}$ (30 ml), dried ($Na_2SO_4$) and evaporated under reduced pressure ($t$ < 35° C). CC ($SiO_2$, EtOAc, 1% $NEt_3$) resulted in **76** as slightly yellow foam (914 mg, 1.42 mmol, 89%).

M.P.: 130 – 133 °C.

$R_f$ ($SiO_2$; EtOAc) 0.28 ($UV_{254}$, $Ce(SO_4)_2$).

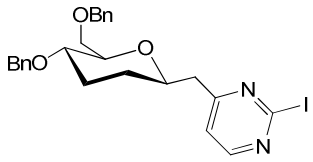
IR (KBr): 3426br. *m*, 3060*w*, 2964*s*, 2937*s*, 2877*m*, 1670*m*, 1607*m*, 1579*m*, 1509*s*, 1488*s*, 1473*m*, 1445*s*, 1383*m*, 1302*w*, 1250*s*, 1178*m*, 1153*w*, 1093*vs*, 1032*m*, 999*w*, 930*w*, 886*w*, 829*w*, 791*w*, 755*w*, 739*w*, 706*w*, 622*s*, 598*w*, 583*w*.

$^{1}$H-NMR (300 MHz, $CD_2Cl_2$): 8.72 (br. *s*, N*H*); 8.50 (*s*, HC(6)); 7.82 (*dd*, *J* = 8.7, 1.5, 2 arom. H); 7.77-7.73 (*m*, 1 arom. H); 7.56-7.36 (*m*, 6 arom. H); 7.31-7.14 (*m*, 6 arom. H); 6.79-6.74 (*m*, 3 arom. H); 4.45 (*d*-like, HC(1')); 3.74-3.68 (*m*, 1H of HC(4'), HC(5'), $H_2C(6')$); 3.71 (s, MeO); 3.71-3.54, 3.47-3.41, 3.36-3.25 (3*m*, 4H of HC(4'), HC(5'), $H_2C(6')$); 2.45 (*s*, $H_3CC_{arom}$); 2.15-2.08, 1.95-1.85, 1.72-1.51 (3*m*, $H_2C(2')$, $H_2C(3')$).

$^{13}$C-NMR (75 MHz, $CD_2Cl_2$): 166.7, 165.3 (2*s*, CO, $NHC_{arom}$); 158.9 (*s*, arom. C); 156.8 (*s*, $CH_3C_{arom}$); 155.8 (*d*, HC(6)); 136.1, 136.0, 134.8 (3*s*, $(CO)C_{arom}$, 2 arom. C); 132.7, 132.1, 130.3, 129.0, 128.8, 128.3, 128.2, 127.6, 127.1, 113.4 (10*d*, arom. C); 81.4, 74.7, 68.3 (3*d*, C(5'), C(1'), C(4')); 65.4 (*t*, C(6')); 55.5 (*q*, OMe); 32.6, 31.0 (2*t*, C(2'), C(3')); 21.9 (*q*, $CH_3$).

ESI-MS: 668.3 (100, $[m+Na]^+$).

**2-iodo-4-[(2',3'-dideoxy-4',6'-bis-*O*-benzyl-$\beta$-D-glucopyranosyl)methyl]-pyrimidine (38)**




To a solution of **34** (100 mg, 0.238 mmol) in 1.7 ml $CH_2I_2$ under $N_2$ was added 0.64 ml (588 mg, 4.77 mmol) isopentylnitrite. The reaction was stirred for 1 h at 85° C, cooled to rt and evaporated

under reduced pressure. CC (3 g $SiO_2$, EtOAc/hexane 1:2) yielded 42 mg (0.0792 mmol, 33%) **38** as a highly viscous oil.

$R_f$ ($SiO_2$; EtOAc/Hexane 1:2) 0.21 ($UV_{254}$, $Ce(SO_4)_2$).

IR(film): 2928*w*, 2864*w*, 1718*s*, 1564*s*, 1527*m*, 1452*m*, 1383*vs*, 1367*vs*, 1327*s*, 1271*m*, 1157*m*, 1097*s*, 739*w*, 699*w*.

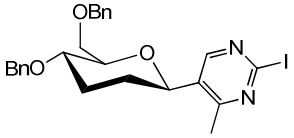
$^1$H-NMR (300 MHz, $CDCl_3$): 8.17 (*d*, *J* = 4.9, $NCH_{arom}$); 7.33-7.23 (*m*, 11 arom. H); 4.61-4.40 (*m*, 4 benz. H); 3.74-3.61 (*m*, HC(1'), $H_2C(6')$); 3.41-3.38 (*m*, HC(5'), HC(4')); 2.94-2.80 (*m*, $C(1')CH_2C_{arom}$); 2.31-2.26 (*m*, 1H of $H_2C(3')$); 1.81-1.76 (*m*, 1H of $H_2C(2')$); 1.56-1.42 (*m*, 2H of $H_2C(3')$, $H_2C(2')$).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 170.4, 157.7, 128.2, 127.5, 127.4, 120.9, 80.4, 75.8, 73.2, 72.9, 70.9, 69.8, 43.4 (*t*, $C(1')CH_2C_{arom}$), 30.5, 29.1 (2*t*, C(3'), C(2')).

ESI-MS: 553.1 (100, $[m+Na]^+$), 569.1 (15, $[m+K]^+$).

HR-ESI-MS: calc. for $C_{25}H_{27}N_2O_3Na_1I_1$ ($[m+Na]^+$) 553.0964; found 553.0958.

**2-iodo-4-methyl-5-(2',3'-dideoxy-4',6'-bis-*O*-benzyl-$\beta$-D-glucopyranosyl)-pyrimidine (39)**




To a solution of 100 mg (0.238 mmol) **35** (dried for 14 h under high vacuum) in 1.7 ml (5.64 g, 21.1 mmol) $CH_2I_2$ under $N_2$ was added at rt 0.64 ml (561 mg, 4.79 mmol) isopentylnitrite and the

reaction heated to 85° C for 1 h. Starting from 60 ° C, the generation of a gas was observed. The reaction was cooled to rt, evaporated and traces of $CH_2I_2$ were removed at 55° C under high vacuum ($p < 10^{-1}$ mbar) over 10 min. CC (5 g $SiO_2$; EtOAc/hexane 1:2) resulted in 45 mg (84.8 µmol, 36%) of **39** as a colourless solid.

$R_f$ ($SiO_2$; EtOAc/Hexane 1:3) 0.15 ($UV_{254}$, $Ce(SO_4)_2$)

IR(KBr): 3443*vs*, 3087*m*, 3062*m*, 3029*m*, 2924*s*, 2865*vs*, 2257*w*, 1658*vs*, 1562*vs*, 1496*s*, 1453*vs*, 1399*s*, 1364*vs*, 1323*vs*, 1261*s*, 1208*s*, 1099*vs*, 1027*s*, 909*w*, 809*w*, 737*vs*, 697*vs*, 609*w*.

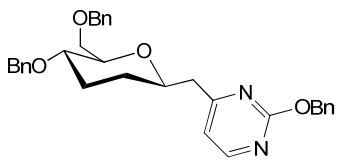
$^1$H-NMR (300 MHz, $CDCl_3$): 8.39 (*s*, $NCH_{arom}$); 7.38-7.23 (*m*, 10 arom. H); 4.65-4.45 (*m*, 5H, 4 benz. H, HC(1')); 3.82-3.68 (*m*, 2H); 3.61 (*ddd*, *J* = 7.4, 5.0, 1.8, 1H); 3.54-3.44 (*m*, 1H), 2.48 (*s*, $CH_3$); 2.44-2.36, 2.15-1.92, 1.71-1.57 (3*m*, $H_2C(3')$, $H_2C(2')$).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 155.9 (*d*, $NCH_{arom}$); 139.5, 132.4 (2*s*, C(1)$C_{arom}$, $H_2CC_{arom}$); 128.3, 128.2, 127.6, 127.5 (4*d*, $CH_{arom}$); 81.3, 74.1, 72.4 (3*d*, C(5'), C(4'), C(1')); 73.4, 71.1, 69.6 (3*t*, C(6'), 2$CH_2C_{arom}$); 30.7, 29.4 (2*t*, C(3'), C(2')); 21.5 (*q*, $CH_3$). N*C*I was not detected.

ESI-MS: 553.1 (100, $[m+Na]^+$).

HR-ESI-MS: calc. for $C_{25}H_{27}N_2O_3Na_1I_1$ ($[m+Na]^+$) 553.0964; found 553.0956.

**2-*O*-benzyl-4-[(2',3'-dideoxy-4,'6'-bis-*O*-benzyl-*β*-D-glucopyranosyl)methyl]-pyrimidine (44)**



A solution of **38** (126 mg, 0.238 mmol) in 8 ml dry $CH_3CN$ (*Fluka*, over molecular sieves), 48 mg (1.20 mmol) NaOH (dried under vacuum $p < 10^{-1}$ mbar, 2 h) and 1.0 ml (1.05 g, 9.52 mmol) BnOH were stirred for 4.5 h at 80° C, cooled to rt, evaporated, dissolved in $CH_2Cl_2$ (20 ml), washed with aq. $NH_4Cl_{sat.}$ (1 x 10 ml), dried ($Na_2SO_4$) and evaporated. CC (3 g $SiO_2$, MeOH/$CH_2Cl_2$ 1:50) yielded 149 mg (0.291 mmol, 79%) **44**.

$R_f$ ($SiO_2$; $CH_2Cl_2$/MeOH 50:1) 0.67 ($UV_{254}$, $Ce(SO_4)_2$)

IR(film): 3686*w* (br.), 3030*s*, 2934*s*, 2862*vs*, 1581*vs*, 1558*vs*, 1496*s*, 1453*vs*, 1409*vs*, 1354*vs*, 1318*s*, 1207*m*, 1085*vs* (br.), 1028*m*, 909*m*, 735*m*, 697*s*.

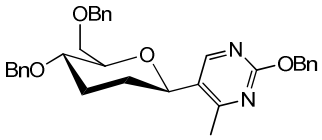
$^1$H-NMR (300 MHz, $CDCl_3$): 8.33 (*d*, *J* = 5.0, $NCH_{arom}$); 7.48-7.18 (*m*, 15 arom. H); 6.91 (*d*, *J* = 5.0, NCHC*H*$_{arom}$); 4.70-4.39 (*m*, 6 benz. H); 3.81-3.61 (*m*, HC(1'), $H_2C$(6')); 3.42-3.38 (*m*, HC(4'), HC(5')); 2.96 (*dd*, *J* = 14.0, 7.5, 1H of C(1')C*H*$_2$C$_{arom}$); 2.78 (*dd*, *J* = 14.0, 5.1, 1H of C(1')C*H*$_2$C$_{arom}$); 2.33-2.24 (*m*, 1H of $H_2C$(3')); 1.81-1.62, 1.48-1.40 (2*m*, 1 H of $H_2C$(3'), $H_2C$(2')).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 170.5 (*s*, $NCO_{arom}$); 158.8 (*d*, $NCH_{arom}$); 140.2, 140.0, 138.8 (3*s*, 3 OCH$_2$*C*$_{arom}$); 128.5, 128.2, 127.9, 127.6, 127.4, 127.3, 126.9 (7*d*, $CH_{arom}$); 115.3 (*d*, NCH*C*H$_{arom}$); 80.5, 76.2, 73.1 (3*d*, C(1'), C(4'), C(5')); 73.2, 71.5, 70.0, 69.2 (4*t*, C(6'), 3 O*C*H$_2$-arom.); 43.8 (*t*, C(1')*C*H$_2$-arom.); 30.6, 29.1 (2*t*, C(2'), C(3')).

ESI-MS: 533.2 (100, $[m+Na]^+$).

HR-ESI-MS: calc. for $C_{32}H_{34}N_2O_4Na_1$ ($[m+Na]^+$) 533.2416; found 533.2416.

**2-*O*-benzyl-4-methyl-5-(2',3'-dideoxy-4',6'-bis-*O*-benzyl-$\beta$-D-glucopyranosyl)-pyrimidine (45)**




A solution of **39** (130 mg, 0.245 mmol) in 8 ml dry $CH_3CN$ (*Fluka*, over molecular sieve), 49 mg (1.225 mmol) NaOH (dried under vacuum $p < 10^{-1}$ mbar, 2 h) and 1.0 ml (1.05 g, 9.52 mmol) BnOH was stirred for 4.5 h at 80° C, cooled to rt, evaporated, dissolved in $CH_2Cl_2$ (20 ml), washed with aq. $NH_4Cl_{sat.}$ (1 x 10 ml), dried ($Na_2SO_4$) and evaporated. CC (3 g $SiO_2$, MeOH/$CH_2Cl_2$ 1:50) yielded 103 mg (0.202 mmol, 82%) **45** as a slightly brown, highly viscous oil.

$R_f$ ($SiO_2$; $CH_2Cl_2$/MeOH 50:1) 0.57 ($UV_{254}$, $Ce(SO_4)_2$).

IR(film): 3062*s*, 3030*s*, 2927*vs*, 2864*vs*, 2199*w*, 1953*w*, 1877*w*, 1587*vs*, 1560*vs*, 1496*s*, 1453*vs*, 1431*vs*, 1356*vs*, 1310*vs*, 1207*s*, 1180*m*, 1099*vs*, 1076*vs*, 910*m*, 815*w*, 796*m*, 736*s*, 697*vs*, 609*w*.

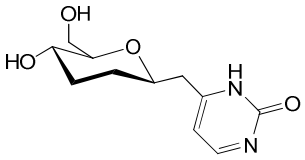
$^1$H-NMR (300 MHz, $CDCl_3$): 8.45 (*s*, $NCH_{arom}$); 7.49-7.46 (*m*, 2 arom. H); 7.37-7.23 (*m*, 13 arom. H); 5.42 (*s*, $NCOCH_2$); 4.65-4.45 (*m*, 4 benz. H); 3.83-3.69 (*m*, 2H); 3.61 (*ddd*, *J* = 11.2, 4.9, 1.9, 1H); 3.56-3.46 (*m*, 1H); 2.48 (*s*, $CH_3$); 2.43-2.35, 2.01-1.92, 1.78-1.59 (3*m*, $H_2C(3')$, $H_2C(2')$).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 156.4 (*d*, $NCH_{arom}$); 128.3, 128.2, 127.9, 127.7, 127.6, 127.5, 127.4 (7*d*, arom. C); 81.3 (*d*); 74.5 (*d*); 73.3(*t*); 72.8 (*d*); 71.1(*t*); 69.8 (*t*); 68.7 (*t*); 30.7, 29.6 (2*t*, C(3'), C(2')); 21.7 (*q*, $CH_3$).

ESI-MS: 533.2 (100, $[m+Na]^+$).

HR-ESI-MS: calc. for $C_{32}H_{34}N_2O_4Na_1$ ($[m+Na]^+$ 533.2416; found 533.2416.

**4-[(2',3'-dideoxy-4,'6'-bis-*O*-benzyl-$\beta$-D-glucopyranosyl)methyl]-pyrimidin-2-one (11)**




To a solution of 7 mg (13.7 µmol) **44** in 1.8 ml dry $CH_2Cl_2$ was added at 0°C 82 µl (82 µmol) of a 1 M $BBr_3$-solution in $CH_2Cl_2$. The reaction was stirred for 3 min at 0° C and quenched by the addition of 2 ml ice-cold water, followed by $NaHCO_{3\ sat.}$ until a pH of about 8 was reached. The phases were separated and the aqueous phase was evaporated under reduced pressure. CC (2 g $SiO_2$, MeOH/$CH_2Cl_2$ 1:1) resulted in 2.6 mg (10.8 µmol, 79%) of **11** as a colourless solid.

IR(KBr): 3442*vs* (br.), 2941*w*, 2868*w*, 2252*w*, 2127*w*, 2091*w*, 1640*s* (br.), 1573*m*, 1456*m*, 1384*w*, 1341*w*, 1262*w*, 1072*m*, 1047*m*, 1025*m*, 995*m*, 827*w*, 767*w*, 594*m*.

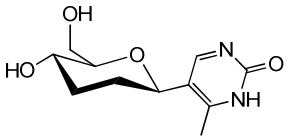
$^1$H-NMR (600 MHz, d$^6$-DMSO): 7.87 (*d*, *J* = 4.9, NC*H*$_{arom}$); 6.01 (*d*, *J* = 4.9, NCHC*H*$_{arom}$); 4.72, 4.44 (2 br. *s*, 2 OH); 3.66-3.57 (*m*, 2H, 1HC(6'), 1HC(1')); 3.42 (*dd*, *J* = 11.4, 5.8, 1HC(6')); 3.22-3.15 (*m*, HC(4')); 3.01 (*ddd*, *J* = 8.8, 5.8, 2.3, HC(5')); 2.59 (*dd*, *J* = 13.4, 6.2, 1H of HC(1')C*H*$_2$-arom.); 2.33 (*dd*, *J* = 13.4, 6.9, 1H of HC(1')C*H*$_2$-arom.); 1.92-1.86 (*m*, H$_{eq}$C(3')); 1.59-1.54 (*m*, H$_{eq}$C(2')); 1.36-1.28 (*m*, H$_{ax}$C(3')); 1.25-1.17 (*m*, H$_{ax}$C(2')).

$^{13}$C-NMR (125 MHz, d$^6$-DMSO): 168.7 (*s*, C(1)CH$_2$*C*$_{arom}$); 156.0 (*d*, NCH$_{arom}$); 103.9 (*d*, NCH*C*H$_{arom.}$); 83.0 (*d*, C(5')); 75.4 (*d*, C(1')); 65.4 (*d*, C(4')); 61.5 (*d*, C(6')); 43.4 (*d*, C(1')); 32.4 (*t*, C(3')); 30.3 (*t*, C(2')).

ESI-MS: 263.0 (100, [m+Na]$^+$).

HR-ESI-MS: calc. for $C_{11}H_{16}N_2O_4Na_1$ ($[m+Na]^+$) 263.1008; found 263.1012.

**4-methyl-5-(2',3'-dideoxy-4',6'-bis-*O*-benzyl-$\beta$-D-glucopyranosyl)-pyrimidin-2-one (37)**




To a solution of 6 mg (11.8 µmol) **45** in 1.5 ml dry $CH_2Cl_2$ was added at 0° C 70 µl (70 µmol) of a 1 M $BBr_3$-solution in $CH_2Cl_2$. The reaction was stirred for 3 min at 0° C and quenched by the addition of 1.5 ml ice-cold water, followed by $NaHCO_{3\ sat.}$ until a pH of about 8 was reached. The phases were separated and the aqueous phase was evaporated under reduced pressure, the resulting colourless solid suspended under ultrasonic condition in $CH_3OH$, filtered, and the $CH_3OH$ was evaporated. CC (2 g $SiO_2$, MeOH:$CH_2Cl_2$ 1:1) resulted in 2.4 mg (9.9 µmol, 84%) of **37** as a colourless solid.

IR(KBr): 3424*vs* (br.), 2955*w*, 2252*w*, 2127*w*, 1646*s* (br.), 1496*w*, 1384*w*, 1318*w*, 1261*w*, 1048*m*, 1025*s*, 997*s*, 827*m*, 767*m*, 634*m*.

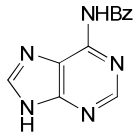
$^1$H-NMR (600 MHz, d$^4$-MeOD): 8.21 (*s*, $HC_{arom}$); 4.46 (*dd*, *J* = 11.1, 1.3, HC(1)); 3.37 (*dd*, *J* = 11.8, 2.4, 1H of $H_2C(6')$); 3.68 (*dd*, *J* = 11.8, 6.0, 1H of $H_2C(6)$); 3.51-3.47 (*m*, HC(4')); 3.37-3.34 (*m*, HC(5')); 2.43 (*s*, $CH_3$); 2.20-2.17 (*m*, $C(3')H_{eq}$); 1.95-1.93 (*m*, $C(2')H_{eq}$); 1.78-1.71 (*m*, $C(2')H_{ax}$); 1.70-1.63 (*m*, $C(3')H_{ax}$).

$^{13}$C-NMR (125 MHz, d$^4$-MeOD): 130.6 (*s*, $H_3CC_{arom}$); 129.0 (*d*, $HC_{arom}$); 120.9 (*s*, $C(1)C_{arom}$); 84.9 (*d*, C(5')); 75.3 (*d*, C(1')); 67.1 (*d*, C(4')); 63.5 (*t*, C(6')); 33.9 (*t*, C(3')); 31.9 (*t*, C(2')); 21.4 (*q*, $CH_3$).

ESI-MS: 263.1 (100, $[m+Na]^+$).

HR-ESI-MS: calc. for $C_{11}H_{16}N_2O_4Na_1$ ($[m+Na]^+$) 263.1008; found 263.1008.

**$N^6$-benzoyladenine**




To a suspension of 10 g (74.0 mmol) adenine in 70 ml pyridine was added at rt 21.5 ml (26.0 g, 185 mmol) BzCl. After stirring for 2 h at 120° C, the pyridine was distilled out under reduced pressure. $NaHCO_{3\ sat.}$ (100 ml) was added, followed by $CHCl_3$ (20 ml), the precipitate was filtered off, washed with $CH_2Cl_2$ (50 ml) and recrystallized from boiling $EtOH/H_2O$ 1:1, resulting in 12.8 g (53.5 mmol, 72%) $N^6$-benzoyladenine as colourless needles.

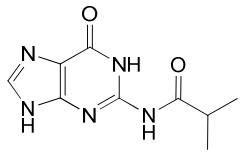
$^1$H-NMR (300 MHz, d$^6$-DMSO): 12.35 (br. *s*, *H*N(9)); 11.62 (br. *s*, *H*NCO); 8.72 (*s*, HC(8)); 8.48 (*s*, HC(2)); 8.12 (*d*, *J* = 7.4, 2 $OCC_qCH_{arom}$); 7.71-7.62, 7.60-7.52 (2*m*, 3H, $HC_{arom}$ of Bz).

$^{13}$C-NMR (75 MHz, d$^6$-DMSO): 171.7 (*s*, CO); 156.2, 151.2 (2*d*, HC(8), HC(2)); 149.6 (*s*); 137.8, 133.7, 133.6 (3*d*, 5 HC of Bz); 119.8 (*s*).

ESI-MS: 262 (69, $[M+Na]^+$); 240 (100, $[M+H]^+$).

Anal. calc. for $C_{12}H_{11}N_5O$ (419.52) C 59.74 H 4.60 N 29.03; found C 60.11 H 4.02 N 29.78.

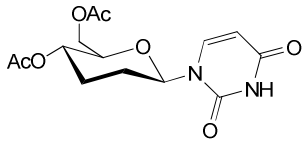
**$N^2$-isobutyrylguanine**



To a suspension of 7.56 g (50.0 mmol) guanine in 100 ml dry DMF (*Fluka*, over molecular sieves) was added at rt 22.4 ml (21.4 g, 135 mmol) isobutyric anhydride. After stirring for 2 h at 150° C the clear solution was cooled to rt and evaporated to 10 ml. The formed precipitate was filtered off, washed with $CH_2Cl_2$ (50 ml) and $NaHCO_{3\ sat.}$ and recrystallized from boiling EtOH/$H_2O$ (1:1), resulting in 9.04 g (42. 6 mmol, 85 %) $N^2$-isobutyrylguanine hydrate as colourless needles.

$^1$H-NMR (300 MHz, d$^6$-DMSO): 13.20, 12.12, 11.56 (3 br. *s*, 3 NH); 8.02 (s, HC(8)); 2.75 (*sept*, *J* = 6.9, *H*C$^i$Pr); 1.11 (*d*, *J* = 6.9, 2 $CH_3$).

**1'-(4',6'-di-*O*-acetyl-2',3'-dideoxy-$\beta$-D-glucopyranosyl)uracil (48)**



To a suspension of 2.83 g (9.75 mmol) **17**, 1.33 g (10.9 mmol) uracil in 120 ml dry $CH_3CN$ was added at rt 2.45 ml (1.86 g, 11.5 mmol) HMDS followed by 1.65 ml (2.07 g, 19.1 mmol) $Me_3SiCl$. The reaction was stirred for 30 min at rt. 1.6 ml (3.55 g, 13.6 mmol) $SnCl_4$ were added and the homogeneous colourless solution was stirred for 16 h at 44° C, cooled to rt and concentrated by evaporation (remaining volume 30 ml). 200 ml EtOAc were added, the reaction was extracted with $H_2O$ (2 x 100 ml), aq. $NaHCO_{3\ sat.}$ (1 x 100 ml), the combined aq. phases were extracted with EtOAc (1 x 150 ml), the combined org. phases were extracted with aq $NaCl_{sat.}$ (1 x 250 ml), dried

($Na_2SO_4$) and evaporated. CC (150 g $SiO_2$, EtOAc/hexane 3:1) resulted in 1.38 g (4.23 mmol, 43%) of pure $\beta$-D-anomer **48**, 210 mg (0.643 mmol, 7%) $\alpha$-D-anomer **49** and 349 mg (1.63 mmol, 17%) enolether **52**.

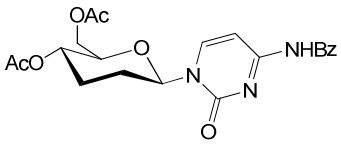
$R_f$ ($SiO_2$; EtOAc) 0.39 ($UV_{254}$, $Ce(SO_4)_2$).

IR(KBr): 3421*w* (br.), 3200*w* (br.), 3100*w*, 2980*w*, 1745*vs* (br.), 1690*vs* (br.), 1630*m*, 1455*m*, 1382*m*, 1317*w*, 1281*s*, 1250*s*, 1197*w*, 1151*w*, 1130*w*, 1122*w*, 1086*m*, 1070*m*, 1042*m*, 997*w*, 931*w*, 820*w*.

$^1$H-NMR (300 MHz, $CDCl_3$): 8.12 (br. *s*, NH); 7.41 (*d*, *J* = 8.1, HC(6)); 5.81 (*d*, *J* = 8.1, HC(5)); 5.74 (*dd*, *J* =10.4, 2.3, HC(1')); 4.74 (*ddd*, *J* = 10.4, 10.2, 4.7, HC(4')); 4.25 (*dd*, *J* = 12.1, 2.3, 1 HC(6')); 4.16 (*dd*, *J* = 2.3, 12.1, 1HC(6')); 3.85 (*ddd*, *J* = 9.9, 6.4, 2.3, HC(5')); 2.38, 2.11 (2*m*, 2H, $H_2C(2')$, $H_2C(3')$); 2.08, 2.07 (2*s*, 2 Ac); 1.74 (*m*, 2H, $H_2C(2')$, $H_2C(3')$).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 170.9, 170.0 (2*s*, Me*C*O); 163,4 (*s*, C(4)); 150.2 (*s*, C(2)); 139.4 (*d*, C(6)); 103.1 (*d*, C(5)); 81.4 (*d*, C(1')); 77.8 (*t*, C(5')); 66.5 (*d*, C(4')); 62.8 (*t*, C(6')); 29.5, 21.0 (2*t*, C(2'), C(3')).

**$N^4$-benzoyl-1-(4',6'-di-*O*-acetyl-2',3'-dideoxy-$\beta$-D-glucopyranosyl)cytosine (50)**




To a suspension of 1.08 g (5.02 mmol) $N^4$-benzoylcytosine in 50 ml dry $CH_3CN$ was added at rt 2,5 ml (2.05 g, 10.1 mmol) *N*,*O*-bis(trimethylsilyl)acetamide. After stirring for 30 min at rt, a

solution of 1.20 g (4.87 mmol) **17** in 25 ml dry $CH_3CN$ was added to the clear solution, followed by 1.5 ml (3.33 g, 12.8 mmol) $SnCl_4$. The reaction was stirred in a preheated oil bath at 49°C for 2 h, evaporated and the residue was dissolved in 250 ml $CHCl_3$, extracted with aq. $NaHCO_{3\ sat.}$ (2 x 150 ml), aq. $NaCl_{sat.}$ (1 x 150 ml). The combined aq. phases were extracted with $CHCl_3$ (1 x 50 ml). The combined org. phases were dried ($Na_2SO_4$) and evaporated. CC (140 g $SiO_2$, $CH_2Cl_2$ (until **52** eluted) to $CH_2Cl_2$/THF 8:1) resulted in 931 mg (2.17 mmol, 45 %) $\beta$-D-anomer **50**, 246 mg (0.573 mmol, 12 %) $\alpha$-D-anomer **51** and 72 mg (0.336 mmol, 7 %) enolether **52**.

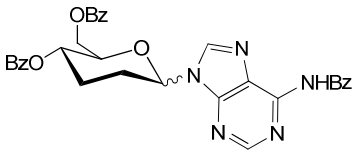
$R_f$ ($SiO_2$; EtOAc) 0.23 ($UV_{254}$, $Ce(SO_4)_2$).

IR(KBr) : 3422*m*, 3242*w*, 3149*w*, 3090*w*, 3072*w*, 3031*w*, 3029*w*, 2961*w*, 2882*w*, 1740*s*, 1700*s*, 1672*s*, 1655*s*, 1629*s*, 1601*m*, 1560*s*, 1487*s*, 1434*m*, 1385*m*, 1377*m*, 1364*m*, 1339*m*, 1308*m*, 1311*s*, 1251*s*, 1191*m*, 1146*m*, 1136*m*, 1100*m*, 1067*s*, 1044*s*, 1003*m*, 961*w*, 933*w*, 897*w*, 873*w*, 860*w*, 799*m*, 784*m*, 709*m*, 701*m*.

$^1$H-NMR (300 MHz, $CDCl_3$): 8.60 (br. *s*, NH); 7.91-7.88 (*m*, HC(6), 2 arom. H); 7.62 (*tt*, *J* = 7.2, 1.3, 1 arom. H); 7.56-.7.48 (*m*, 2 arom H); 6.98 (br. *s*, HC(5)); 5.85 (*dd*, *J* = 10.3, 2.0, HC(1')); 4.78 (*dt*, *J* = 10.5, 4.7, HC(4')); 4.29 (*dd*, *J* = 12.2, 5.6, 1 HC(6')); 4.19 (*dd*, *J* = 12.2, 2.2, 1 HC(6')); 3.88 (*ddd*, *J* = 10.0, 5.6, 2.2, HC(5')); 2.41-2.29 (*m*, 2H, HC(2'), HC(3')); 2.09, 2.08 (2*s*, 2Ac); 1.84-1.70, 1.68-1.54 (2*m*, 2H, HC(2'), HC(3')).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 170.8, 169.8 (2*s*, CO), 166.7 (*s*, CO); 162.3 (*s*, C(4)); 154.2 (*s*, C(2)); 144.0 (*d*, C(6)); 133.0 (*s*, arom. C); 133.4, 129.0, 127.5 (3*d*, 3 arom. C); 97.1 (*d*, C(5)); 83.1 (*d*, C(1')); 78.0 (*d*, C(5')); 66.8 (*d*, C(4')); 62.8 (*t*, C(6')); 30.4, 27.9 (2*t*, C(2'), C(3')); 21.1, 20.8 (2*q*, 2*Me*CO).

**$N^6$-benzoyl-9-(4',6'-di-*O*-benzoyl-2',3'-dideoxy-$\alpha/\beta$-D-glucopyranosyl)adenine (53$\alpha$/53$\beta$)**



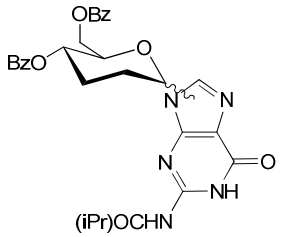

A mixture of 4.30 g (11.61 mmol) **22** and 2.78 g (11.59 mmol) $N^6$-benzoyladenine was dried under high vacuum ($p < 10^{-1}$ mbar) at rt for 4 h and suspended under $N_2$ in 170 ml dry $CH_3CN$. 1.93 ml (9.25 mmol) HMDS, 1.17 ml (9.28 mmol) $Me_3SiCl$ and 2.05 ml (17.40 mmol) $SnCl_4$ were added. The yellow homogeneous solution was allowed to stand for 16 h, before 1.36 ml (11.59 mmol) of additional $SnCl_4$ was added while stirring and the reaction was stirred for 4 h at rt and worked up after a total reaction time of 20 h. $NaHCO_{3\ sat.}$ (100ml) was added, the $CH_3CN$ was evaporated under reduced pressure and the residue was extracted with EtOAc (2 x 150 ml), the combined organic phases were extracted with $NaCl_{sat.}$ (250 ml), dried ($MgSO_4$) and evaporated. The resulting white foam was chromatographed (250 g $SiO_2$, EtOAc) resulting in 3.56 g (6.16 mmol, 53%) as a mixture of anomers (**53$\beta$/53$\alpha$** = 74:26 ($^1$H-NMR)).

$R_f$ ($SiO_2$; EtOAc) 0.20 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, $CDCl_3$): 8.94 (br. *s*, NH); 8.81 (*s*, HC(2), 2.8H, $\beta$-D); 8.78 (br. *s*, HC(2), 1.0H, $\alpha$-D); 8.30 (*s*, HC(8), 1.1H, $\alpha$-D); 8.24 (*s*, HC(8), 2.9H, $\beta$-D); 8.08-7.98 (*m*, arom. H); 7.83-7.80 (*m*, arom. H); 7.65-7.37 (*m*, arom. H); 6.26 (*dd*, *J* = 5.5, 4.0, HC(1'), $\alpha$-D); 6.05 (*dd*, *J* = 10.6, 2.8, HC(1'), $\beta$-D); 5.25 (*ddd*, *J* = 10.3, 4.8, HC(4'), $\beta$-D); 4.71 (*dd*, *J* = 12.0, 7.1, HC(4'), $\alpha$-D); 4.68 (*dd*, *J* = 12.2, 2.6, $\alpha$-D); 4.56 (*dd*, *J* = 12.0, 3.7, $\alpha$-D); 4.47 (*dd*, *J* = 12.2, 5.6, 1HC(6'), $\beta$-D); 4.37-4.27 (*m*, HC(5'), $\alpha$-D, $\beta$-D); 2.71-2.73 (*m*, 1 HC(3'), $\alpha$-D, $\beta$-D); 2.48-2.30 (*m*, 2HC(2'); $\alpha$-D, $\beta$-D); 2.07-1.94 (*m*, 1HC(3'), $\alpha$-D, $\beta$-D).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 166.3, 165.4, 164.7 (3*s*, CO, *β*-D); 152.6 (*d*, C(2), *β*-D); 151.2 (*s*, C(6), *β*-D); 149.8 (*s*, C(4), *β*-D); 140.4 (*d*, C(8), *β*-D); 133.7, 129.6, 129.4 (3*s*, arom. C, *β*-D); 133.6, 133.2, 132.9, 129.7, 128.8, 128.6, 128.3, 128.0 (6*d*, arom. C, *β*-D); 122.8 (*s*, C(5), *β*-D); 81.8 (*d*, C(1'), *β*-D); 77.9 (*d*, C(5'), *β*-D); 67.6 (*d*, C(4'), *β*-D); 63.4 (*t*, C(6'), *β*-D); 30.6, 28.5 (2*t*, C(2'), C(3'), *β*-D).

**7- and 9-(4',6'-di-*O*-benzoyl-2',3'-dideoxy-*α*/*β*-D-glucopyranosyl)$N^2$-isobutyrylguanine (54*β*/54*α*/55*β*)**

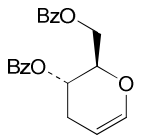



To a suspension of 2.15 g (8.99 mmol) $N^2$-isobutyrylguanine hydrate in 20 ml refluxing $CH_3CN$ was added under $N_2$ 10.8 ml (44.2 mmol) *N*,*O*-bis(trimethylsilyl)acetamide and the temperature was reduced to 75° C. To the clear solution was added after 30 min, 1.60 g (4.32 mmol) **22** in 20 ml dry $CH_3CN$ and 2.0 ml (11.0 mmol) $CF_3SO_3SiMe_3$. The reaction was cooled to rt after 20 h under reflux and 30 ml $CH_3CN$ were removed under reduced pressure. The crude reaction was taken up in $CH_2Cl_2$ (200 ml), 100 ml $NaHCO_{3\ sat.}$ was added and the solution stirred for 15 min. The resulting suspension was filtered over Celite, the organic phase was separated, the aqueous phase was extracted with $CH_2Cl_2$ (2 x 100 ml), the combined organic phases were extracted with $NaHCO_{3\ sat.}$ (2 x 100 ml), dried ($Na_2SO_4$) and evaporated. CC (75 g $SiO_2$, $CH_2Cl_2/CH_3OH$ 93:7) resulted in enolether **56**, followed by 894 mg (1.60 mmol, 37%, $N^9$-*β*/$N^9$-*α*/$N^7$-*β* = 80:14:6 ($^1$H-NMR)) **54*β*/54*α*/55*β*** as a slightly yellow foam.

$R_f$ ($SiO_2$; $CH_2Cl_2$/MeOH 93 :7) 0.43 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, $CDCl_3$): 11.97 (br. *s*, NH); 8.62 (br. *s*, NH); 8.26 (br. *s*, NH); 8.05-7.85 (*m*, HC(8), arom. H); 7.61-7.36 (*m*, arom. H); 6.20 (*dd*, HC(1'), $N^7$-*β*); 6.00 (*dd*, HC(1'), $N^9$-*α*); 5.65 (*dd*, *J* = 10.9, 2.5, HC(1'), $N^9$-*β*); 5.28-5.22 (*m*, HC(4')); 4.70-4.61 (*m*, HC(6')); 4.51-4.40 (*m*, HC(6')); 4.27-4.17 (*m*, HC(5')); 2.66-2.56 (*m*, $Me_2CHCO$); 2.42-1.80 (*m*, HC(2'), HC(3')); 1.26 (*d*, *J* = 6.9, $Me_2CHCO$); 1.24 (*d*, $Me_2CHCO$); 1.22 (*d*, $Me_2CHCO$).

**(2*R*, 3*S*)-2-(benzoylmethoxy)-3-(benzoylhydroxy)-2*H*,3*H*-dihydropyrane (56)**



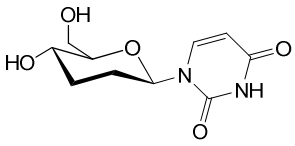

$R_f$ ($SiO_2$; $CH_2Cl_2$/MeOH 93 :7) 0.80 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, $CDCl_3$): 8.06-8.02 (*m*, 4 arom H.); 7.59-7.51 (*m*, 2 arom. H); 7.46-7.39 (*m*, 4 arom. H); 6.42 (*dt*, *J* = 6.1, 2.0, HC(1)); 5.43-5.36 (*m*, HC(2)); 4.77 (*ddd*, *J* = 7.6, 4.5, 3.1, HC(4)); 4.65 (*dd*, *J* = 11.9, 3.5, 1H of $H_2C(6)$); 4.51 (*dd*, *J* = 11.9, 5.5, 1H of $H_2C(6)$); 4.41-4.35 (*m*, HC(5)); 2.63 (*dddd*, *J* = 17.1, 6.0, 4.5, 0.8, 1H of $H_2C(3)$); 2.32-2.22 (*m*, 1H of $H_2C(3)$).

CI-MS: 104.9 (61), 93.9 (100), 80.9 (82), 76.9 (37), 27.9 (7).

ESI-MS: 361.1 (100, $[m+Na]^+$).

**1-(2',3'-dideoxy-β-D-glucopyranosyl)uracil (57)**



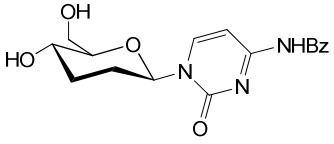
A solution of 1.37 g (4.20 mmol) **48** in 17 ml conc. $NH_3$ in $CH_3OH$ was allowed to stand at rt for 15 h. After evaporating and drying under high vacuum ($p < 10^{-1}$ mbar) 925 mg (3.82 mmol, 91%) **57** was obtained as a colourless foam.

$R_f$ ($SiO_2$; EtOAc/MeOH 5:1) 0.20 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, d$^6$-DMSO): 11.24 (br. *s*, NH); 7.71 (*d*, *J* = 8.1, HC(6)); 5.61 (*d*, *J* = 8.1, HC(5)); 5.51 (*dd*, *J* = 7.9, 5.1, HC(1')) ; 4.87 (*d*, *J* = 5.2, HOC(4')); 4.51 (*t*, *J* = 5.8, HOC(6')); 3.68 (*ddd*, *J* = 11.8, 5.2, 1.7, 1H of $H_2C(6')$); 3.48 (*m*, 1H of $H_2C(6')$); 3.39-3.16 (*m*, HC(4'), HC(5')); 2.06-1.96, 1.78-1.71, 1.61-1.40 (3*m*, $H_2C(2')$, $H_2C(3')$).

$^{13}$C-NMR (75 MHz, d$^6$-DMSO): 162.8 (*s*, C(4)); 149.8 (*s*, C(2)); 140.76 (*d*, C(6)); 101.5 (*d*, C(5)); 83.4 (*d*, C(1')); 80.8 (*d*, C(5')); 63.9 (*d*, C(4')); 61.0 (*t*, C(6')); 31.3, 28.8 (2*t*, C(2'), C(3')).

**$N^4$-benzoyl-1-(2',3'-dideoxy-β-D-glucopyranosyl)cytosine (58)**



To a solution of 881 mg (2.05 mmol) **50** in 82 ml THF/MeOH/$H_2O$ 5:4:1 at 0° C was added 8.2 ml aqueous 2 N NaOH. The reaction was stirred for 25 min at 0°C, 1.05 g (19.7 mmol) $NH_4Cl$ was

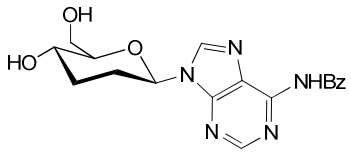
added as a solid and the reaction was stirred for 30 min at 0° C. The reaction was evaporated to a volume of 10 ml, during which a solid was formed. To complete the precipitation the reaction mixture was stored for 50 h at 4° C, the precipitate was filtered and washed with ice water (10 ml) and dried under vacuum, resulting in 695 mg (2.01 mmol, 98%) **58** as a white solid.

$R_f$ ($SiO_2$; $CH_2Cl_2$/MeOH 9:1) 0.22 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, d$^6$-DMSO): 8.21 (*d*, *J* = 7.5, HC(6)); 8.01 (*dd*, *J* = 7.1, 1.4, 2 arom. H); 7.63 (*tt*, *J* = 7.4, 1.3, 1 arom. H); 7.51 (*t*, *J* = 7.4, 2 arom. H); 7.34 (*d*, *J* = 7.3, HC(5)); 5.64 (*dd*, *J* = 10.0, 1.9, HC(1')); 4.95 (*d*, *J* = 5.2, HOC(4')); 4.56 (*t*, *J* = 5.9, HOC(6')); 3.71 (*ddd*, *J* = 11.7, 5.2, 1.4, 1H of $H_2C$(6')); 3.58-3.50 (*m*, 1H of $H_2C$(6')); 3.47-3.32 (*m*, HC(4'), HC(5')); 2.07-2.03, 1.97-1.91, 1.71-1.50 (3*m*, $H_2C$(2'), $H_2C$(3')).

$^{13}$C-NMR (75 MHz, d$^6$-DMSO): 167.3 (*s*, CO); 162.8 (*s*, C(4)); 153.6 (*s*, C(2)); 145.4 (*d*, C(6)); 133.0 (*s*, arom. C); 132.6, 128.4 (2*d*, arom. C); 96.3 (*d*, C(5)); 83.4 (*d*, C(1')); 82.2 (*d*, C(5')); 63.8 (*d*, C(4')); 60.9 (*t*, C(6')); 31.2, 29.8 (2*t*, C(2'), C(3')).

**$N^6$-benzoyl-9-(2',3'-dideoxy-$\beta$-D-glucopyranosyl)adenine (59)**




To a solution of 1 g (1.73 mmol) **53$\beta$**/**53$\alpha$** in 100 ml THF/MeOH/$H_2O$ 5:4:1 was added at 0° C 10 ml 2 N NaOH. The reaction was stirred for 20 min at 0° C. 1.28 g (24.0 mmol) $NH_4Cl$ was added and the reaction mixture was evaporated. The remaining solid was dissolved in MeOH, 1 g $SiO_2$ was added, evaporated and chromatographed (15 g $SiO_2$, $CH_2Cl_2$/MeOH 8:1), resulting in 522 mg

(1.41 mmol, 82 %) of a diastereomeric mixture ($\beta$-D/$\alpha$-D 2.3: 1 (according to $^1$H-NMR)). The diastereomeric mixture was separated by recrystallisation from 35 ml boiling MeOH resulting in 311 mg (0.842 mmol, 70 %) pure $\beta$-D-anomer **59** as colourless needles.

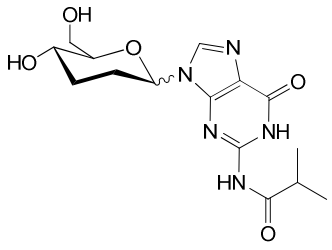
$R_f$ ($SiO_2$; $CH_2Cl_2$/MeOH 8:1) 0.17 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, d$^6$-DMSO): 11.16 (br. *s*, NH); 8.77 (*s*, HC(2)); 8.70 (*s*, HC(8)); 8.04 (*d*, *J* = 7.1, 2 arom. H); 7.68-7.62 (*m*, 1 arom. H); 7.58-7.52 (*m*, 2 arom. H); 5.87 (*dd*, *J* = 10.7, 1.5, HC(1')); 4.96 (*d*, *J* = 5.0, HOC(4')); 4.56 (*dd*, *J* = 5.9, HOC(6')); 3.70 (*dd*, *J* = 10.8, 6.0, 1H of $H_2$C(6')); 3.53-3.43 (*m*, HC(4'), HC(5'), 1H of $H_2$C(6')); 2.51-2.40, 2.18-2.07, 1.77-1.62 (3*m*, $H_2$C(2'), $H_2$C(3')).

$^{13}$C-NMR (75 MHz, d$^6$-DMSO): 165.5 (*s*, CO); 151.8 (*s*, C(6)); 151.8 (*d*, C(2)); 150.2 (*s*, C(4)); 142.6 (*d*, C(8)); 133.2 (*s*, arom. C); 132.3, 128.3 (2*d*, arom. C); 125.4 (*s*, C(5)); 83.3 (*d*, C(1')); 80.7 (*d*, C(5')); 64.1, (*d*, C(4')); 60.9 (*t*, C(6')); 31.4, 29.0 (2*t*, C(2'), C(3')).

ESI-MS: 408.2 (41, $[m+K]^+$); 392.2 (12, $[m+H]^+$); 370.2 (100, $[m+H]^+$).

**$N^2$-isobutyryl-9-(2',3'-dideoxy-$\alpha$/$\beta$-D-glucopyranosyl)guanine (60$\alpha$/60$\beta$)**




To a solution of 185 mg (0.331 mmol) **54$\beta$**/**54$\alpha$**/**55$\beta$** in 14 ml THF/MeOH/$H_2O$ 5:4:1 was added at 0° C 1.3 ml 2 N aq. NaOH. The reaction was stirred for 25 min at 0° C, then 170 mg (3.18 mmol)

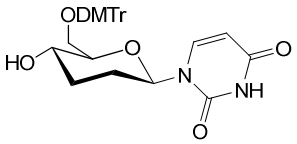
$NH_4Cl$ was added and the reaction was stirred for 60 min at 0° C. The reaction mixture was evaporated. The remaining solid was dispersed in 5 ml MeOH, 1 g $SiO_2$ was added, the material was concentrated and chromatographed (5 g $SiO_2$, $CH_2Cl_2$/MeOH 5:1), resulting in 83 mg (0.236 mmol, 76%) of **60*β*/60*α*** (**60*β*/60*α*** = 6.3:1 ($^1$H NMR)) as a white foam.

$R_f$ ($SiO_2$; $CH_2Cl_2$/MeOH 5:1) 0.25 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, d$^6$-DMSO): 12.08 (br. *s*, NH); 11.73 (br. *s*, NH); 8.22 (*s*, HC(8), *β*-D, 1.00 H); 8.18 (*s*, HC(8), *α*-D, 0.16 H); 5.91 (*t*, *J* = 4.7, HC(1'), *α*-D, 0.17 H); 5.56 (*dd*, *J* = 11.1, 2.0, HC(1'), *β*-D, 0.98 H); 4.97 (br. *s*, HOC(4'), *β*-D, 0.99 H) ; 4.88 (br. *s*, HOC(4'), *α*-D, 0.16 H); 4.58 (br. *s*, HOC(6')); 3.72-3.29 (*m*, HC(4'), $H_2C$(6')); 3.19-3.15 (*m*, HC(5')); 2.80 (*s*, *J* = 6.7, $Me_2CHCO$); 2.36-2.22 (*m*, 1H of $H_2C$(2'), $H_2C$(3')); 2.15-1.94 (*m*, 2H of $H_2C$(2'), $H_2C$(3')); 1.67-1.53 (*m*, 1H of $H_2C$(2'), $H_2C$(3')); 1.13 (*d*, *J* = 6.9, $Me_2CHCO$).

$^{13}$C-NMR (75 MHz, d$^6$-DMSO): 180.1 (*s*, CO); 154.7 (*s*, C(6)); 148.0 (*s*, C(2), C(4)); 137.4 (*d*, C(8)); 119.8 (*s*, C(5)); 83.4 (*d*, C(1')); 80.4 (*d*, C(5')); 63.9 (*d*, C(4')); 60.7 (*t*, C(6')); 34.6 (*d*, $Me_2CHCO$); 34.6, 31.5 (2*t*, C(2'), C(3')); 18.74, 18.66 (2*q*, $Me_2CHCO$).

**1-{2',3'-dideoxy-6'-*O*-[(4,4'-dimethoxytriphenyl)methyl]-*β*-D-glucopyranosyl}uracil (61)**




538 mg (2.22 mmol) **57**, 988 mg (2.92 mmol) $(MeO)_2$TrCl and 12 mg (98.2 µmol) 4-(dimethylamino)pyridine were dried under high vacuum ($p < 10^{-1}$ mbar) for 10 min at rt. 7.7 ml pyridine, followed by 0.5 ml $NEt_3$, was added and the red solution was stirred for 3 h, 20 min at rt.

1.9 ml MeOH were added and the solvent was evaporated at 30° C. The crude product was dissolved in 150 ml $CH_2Cl_2$, and extracted with aq. $NaCl_{sat.}$ (3 x 80 ml). The combined aqueous phases were extracted with $CH_2Cl_2$ (1 x 40 ml). The combined org. phases were dried ($Na_2SO_4$), evaporated ($t$ = 30 ° C), and dried for 10 min under high vacuum ($p < 10^{-1}$ mbar). CC (50 g $SiO_2$; EtOAc/hexane 1:1 to EtOAc) resulted in 983 mg (1.81 mmol, 81%) **61** as a colourless foam.

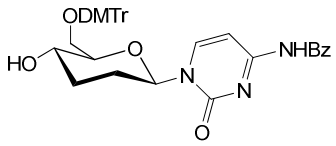
$R_f$ ($SiO_2$; EtOAc) 0.24 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, d$^6$-DMSO): 11.39 (br. *s*, NH); 7.70 (*d*, *J* = 8.1, HC(6)); 7.40 (*d*, *J* = 6.9, 2 arom. H); 7.28-7.20 (*m*, 7 arom. H); 6.84 (*dd*, *J* = 8.9, 1.6, 4 arom. H); 5.72 (*d*, *J* = 8.1, HC(5)); 5.62 (*m*, HC(1')); 4.83 (*d*, *J* = 6.0, HOC(4')); 3.72 (*s*, 2 OMe); 3.57 (*m*, HC(5')); 3.25-3.22 (*m*, HC(4'), 1H of $H_2C(6')$); 3.08 (*dd*, *J* = 10.0, 6.3, 1H of $H_2C(6')$); 2.05-1.99 (*m*, 1H of $H_2C(2')$, $H_2C(3')$); 1.84-1.78 (*m*, 2H of $H_2C(2')$, $H_2C(3')$); 1.61-1.53 (*m*, 1H of $H_2C(2')$, $H_2C(3')$).

$^{13}$C-NMR (75 MHz, d$^6$-DMSO): 162.8 (*s*, C(4)); 157.7 (*s*, arom. C); 150.0 (*s*, C(2)); 145.0 (*s*, arom. C); 140.4 (*d*, C(6)); 135.8, 135.7 (2*s*, arom. C); 129.7, 127.7, 127.5, 127.4, 112.9 (5*d*, arom. C); 101.5 (*d*, C(5)); 85.0 (*s*, *C*OC(6')); 81.5, 80.8 (2*d*, C(1'), C(5')); 64.2 (*d*, C(4')); 63.9 (*t*, C(6')); 54.9 (*q*, MeO); 31.5, 28.6 (2*t*, C(2'), C(3')).

ESI-MS: 567.4 (100, $[m+Na]^+$); 303.2 (4).

**$N^4$-benzoyl-1-{2',3'-dideoxy-6'-*O*-[(4,4'-dimethoxytriphenyl)methyl]-$\beta$-D-glucopyranosyl}cytosine (62)**



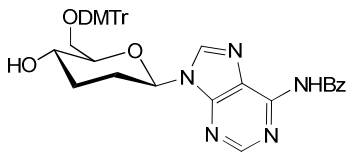
456 mg (1.32 mmol) **58** was suspended in 14 ml pyridine and dissolved under heating to 115° C. Careful cooling to rt resulted in a clear solution to which 542 mg (1.59 mmol) $Bu_4NClO_4$ and 537 mg (1.58 mmol) $(MeO)_2TrCl$ was added. After stirring for 1 h at rt, 0.7 ml MeOH was added and the reaction was stirred for another 10 min at rt, evaporated ($t$ = 30° C), dried under high vacuum ($p < 10^{-1}$ mbar), dissolved in 60 ml $CH_2Cl_2$ and extracted with aq. $NaHCO_{3\ sat.}$ (3 x 30 ml). The combined aq. phases were extracted with $CH_2Cl_2$ (2 x 20 ml) and the combined org. phases were dried ($Na_2SO_4$) and evaporated ($t$ = 30° C). CC (30 g $SiO_2$, EtOAc to EtOAc/EtOH 50:1) resulted in 593 mg (0.92 mmol, 69%) of **62**.

$R_f$ ($SiO_2$; EtOAc) 0.08 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, $CD_2Cl_2$): 8.63 (br. *s*, NH); 7.93 (br.*s*, 2 arom. H, HC(6)); 7.61 (*tt*, *J* = 7.4, 1.5, 1 arom. H); 7.52-7.42 (*m*, HC(5), 4 arom. H); 7.34-7.20 (*m*, 7 arom. H); 6.86-6.82 (*m*, 4 arom. H); 5.76 (*dd*, *J* = 10.4, 2.0, HC(1')); 3.792 (*s*, MeO); 3.790 (*s*, MeO); 3.75-3.60 (*m*, HC(4'), HC(5')); 3.44 (*dd*, *J* = 10.0, 4.3, 1H of $H_2C(6')$; 3.45 (*dd*, *J* = 10.0, 4.5, 1H of $H_2C(6')$); 2.25-2.17 (*m*, 2 H of $H_2C(2')$, $H_2C(3')$); 1.79-1.63 (*m*, 1H of $H_2C(2')$, $H_2C(3')$); 1.58-1.46 (*m*, 1H of $H_2C(2')$, $H_2C(3')$).

ESI-MS: 670.3 (40, $[m+Na]^+$); 242.3 (100).

**$N^6$-benzoyl-9-{2',3'-dideoxy-6'-*O*-[(4,4'-dimethoxytriphenyl)methyl]-$\beta$-D-glucopyranosyl}adenine (63)**



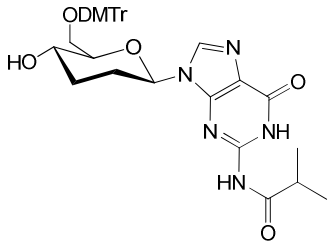
277 mg (0.750 mmol) **59**, 309 mg (0.904 mmol) $Bu_4NClO_4$ and 308 mg (0.909 mmol) $(MeO)_2TrCl$ were dried under high vacuum ($p < 10^{-1}$ mbar) for 2 h, dissolved in 3.7 ml pyridine and stirred for 80 min at rt. The reaction mixture was evaporated, dissolved in 50 ml $CH_2Cl_2$, extracted with aq. $NaHCO_{3\ sat.}$ (2 x 20 ml) and aq. $NaCl_{sat.}$ (1 x 20 ml). The combined aqueous phases were extracted with $CH_2Cl_2$ (2 x 20 ml) and the combined org. phases were dried ($Na_2SO_4$) and evaporated. CC (20 g $SiO_2$, $CH_2Cl_2$/acetone 3:1 to $CH_2Cl_2$/acetone 1:1) resulted in 458 (0.682 mmol, 91%) **63** as a colourless foam.

$R_f$ ($SiO_2$; $CH_2Cl_2$/acetone 3:1) 0.18 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, d$^6$-DMSO): 11.20 (br. *s*, NH); 8.80 (*s*, HC(2)); 8.70 (*s*, HC(8)); 8.05 (*d*, *J* = 7.5, 2 arom. H); 7.67-7.61 (*m*, 1 arom. H); 7.58-7.53 (*m*, 2 arom. H); 7.37-7.34 (*m*, 2 arom. H); 7.23-7.16 (*m*, 7 arom. H); 6.74-6.71 (*m*, 4 arom. H); 5.96 (*dd*, *J* = 10.2, 2.2, HC(1')); 4.92 (*d*, *J* = 6.0, HOC(4')); 3.75-3.69 (*m*, HC(4')); 3.70 (*s*, MeO); 3.69 (*s*, MeO); 3.47-3.40 (*m*, HC(5')); 3.29-3.25 (*m*, 1H of $H_2C$(6')); 3.06 (*dd*, *J* = 10.2, 6.9, 1H of $H_2C$(6')); 2.55-2.45, 2.17-2.09, 1.78-1.65 (3*m*, $H_2C$(2'), $H_2C$(3')).

ESI-MS: 694.3 (100, $[m+Na]^+$).

**9-{2',3'-dideoxy-6'-*O*-[(4,4'-dimethoxytriphenyl)methyl]-*β*-D-glucopyranosyl}-$N^2$-isobutyrylguanine (64)**



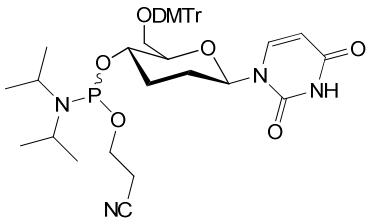
To a solution of 55 mg (0.157 mmol) of **60β/60α** ($\beta/\alpha$ = 6.8:1, ($^1$H-NMR)) in 1.5 ml pyridine was added 63 mg (0.184 mmol) $Bu_4NClO_4$ and 63 mg (0.186 mmol) $(MeO)_2TrCl$ and the reaction was stirred for 2 h at rt. To the solution was added 0.1 ml MeOH and the reaction was stirred for another 15 min, evaporated, coevaporated with 2 ml toluene (to remove traces of pyridine), dissolved in 10 ml $CH_2Cl_2$ and extracted with $NaHCO_{3\ sat.}$ (3 x 5 ml). The combined aq. phases were extracted with $CH_2Cl_2$ (3 x 5 ml) and the combined org. phases were dried ($Na_2SO_4$) and evaporated. CC (10 g $SiO_2$, EtOAc/EtOH 19:1) resulted in 56 mg (85.7 μmol, 63%) pure **64β** as colourless foam.

$R_f$ ($SiO_2$; EtOAc/EtOH 19:1) 0.15 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, d$^6$-DMSO): 12.24 (br. *s*, NH); 11.64 (br. *s*, NH); 8.22 (*s*, HC(8)); 7.37 (*dd*, *J* = 7.7, 1.6, 2 arom. H); 7.28-7.16 (*m*, 7 arom. H); 6.81 (*dd*, *J* = 8.8, 6.9, 4 arom. H); 5.68 (*dd*, *J* = 11.3, 1.7, HC(1')); 4.97 (*d*, *J* = 6.0, HOC(4')); 3.743 (*s*, MeO); 3.739 (*s*, MeO); 3.67-3.62 (*m*, 1H of $H_2C(6')$); 3.48-3.29 (*m*, HC(4'), HC(5')); 3.11 (*dd*, *J* = 10.0, 6.7, 1H of $H_2C(6')$); 2.84 (*sept.*, *J* = 6.8, $Me_2CHCO$); 2.41-2.28, 2.18-2.07, 1.72-1.59 (3*m*, $H_2C(2')$, $H_2C(3')$); 1.12 (*d*, *J* = 6.8, 3H, $Me_2CHCO$); 1.11 (*d*, *J* = 6.8, 3H, $Me_2CHCO$).

$^{13}$C-NMR (75 MHz, d$^6$-DMSO): 180.1 (*s*, CO); 154.7 (*s*, C(6)); 148.1 (*s*, C(2), C(4)); 137.3 (*d*, C(8)); 157.8, 144.9, 135.6 (3*s*, 3 arom. C); 120.0 (*s*, C(5)); 129.7, 127.7, 127.5, 126.4, 112.8 (5*d*, arom. C); 85.0 (*s*, $COC(6')$); 81.6 (*d*, C(1')); 80.4 (*d*, C(5')); 64.3 (*d*, C(4')); 63.9 (*t*, C(6')); 54.8 (*q*, MeO); 34.6 (*d*, $Me_2CHCO$); 31.8, 29.2 (2*t*, C(2'), C(3')); 18.8, 18.7 (2*q*, $Me_2CHCO$).

**1-{4'-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-2',3'-dideoxy-6'-*O*-[(4,4'-dimethoxytriphenyl)methyl]-$\beta$-D-glucopyranosyl}uracil (65)**




After drying for 4.5 h under high vacuum ($p < 10^{-1}$ mbar) 215 mg (0.395 mmol) **61** was suspended under Ar at rt in 6 ml dry $CH_2Cl_2$ and 0.27 ml (1.55 mmol) $(iPr)_2EtN$ (bp 128° C, distilled over $CaH_2$) and 0.14 ml (0.608 mmol) chloro(2-cyanoethoxy)(diisopropylamino)phosphine was added dropwise. During the addition of the phosphine, the reaction became clear. After stirring for 1 h at rt, the clear solution was diluted with EtOAc (100 ml), extracted with $Na_2CO_{3sat}$. (2 x 50 ml) and $NaCl_{sat.}$ (40 ml), dried ($Na_2SO_4$) and evaporated. CC (10 g $SiO_2$, EtOAc/hexane 2:1, crude material applied in $CH_2Cl_2$) and reprecipitation from 1 ml $CH_2Cl_2$ with 30 ml hexane resulted in 247 mg (0.331 mmol, 84%) **65** as a colourless foam in a 1:1-diastereomeric mixture ($^1$H- and $^{31}$P-NMR).

$R_f$ ($SiO_2$; EtOAc) 0.52, 0.48 ($UV_{254}$, $Ce(SO_4)_2$).

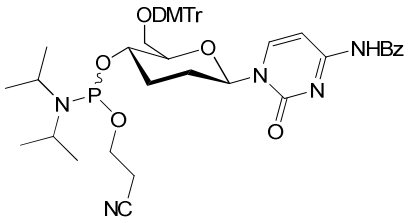
$^1$H-NMR (300 MHz, $CDCl_3$): 9.08 (br. *s*, NH); 7.55-7.51, 7.48-7.44, 7.35-7.29 (3*m*, 9 arom. H); 6.82-6.77 (*m*, 4 arom. H); 5.83, 5.81 (2*d*, *J* = 3.0, 1H, HC(6)); 5.78-5.71 (*m*, HC(1')); 3.96-3.92 (*m*, 1H, OC*H*$_2$CH$_2$CN); 3.79, 3.78, 3.77, 3.76 (4*s*, 6H, MeO); 3.75-3.72 (*m*, 1H, HC(4')); 3.72-3.57 (*m*, 1H, OC*H*$_2$CH$_2$CN); 3.57-3.23 (*m*, 5H, HC(5'), HC(6'), Me$_2$C*H*N); 2.59 (*t*, *J* = 6.1, 1H, OCH$_2$C*H*$_2$CN); 2.41-2.32 (*m*, 1H, HC(2'), HC(3')); 2.30 (*dt*, *J* = 6.2, 3.6, 1H, OCH$_2$CH$_2$CN); 2.09-2.04 (*m*, 1H, HC(2'), HC(3')); 1.80-1.62 (*m*, 2H, HC(2'), HC(3')); 1.13, 1.09, 1.07 (3*d*, *J* = 6.7, *Me*$_2$CHN); 0.89 (*m*, 3H, *Me*$_2$CHN).

$^{13}$C-NMR (75 MHz, $CDCl_3$): 163.1 (*s*, C(4)); 158.4, 158.3 (2*s*, arom. C); 149.7 (*s*, C(2)); 144.9, 144.7 (2*s*, 2 arom. C); 139.7, 139.6 (2*d*, C(6)); 136.1, 136.0, 135.9, 130.2, 130.1, 128.4, 128.3, 127.6, 126.7, 126.6, 112.9 (11*d*, arom. C); 117.6, 117.5 (2s, CN); 102.4, 102.3 (2*d*, C(5)); 85.7 (*s*, *C*-O-C(6')); 81.7 (*d*, C(1')); 81.5, 81.4 (2*d*, C(5')); 67.4 (*dd*, $J_P$ = 13.0, C(4')); 66.9 (*dd*, $J_P$ = 16.6, C(4')); 63.1, 63.0 (2*t*, C(6')); 58.3, 57.3 (O*C*$H_2$C$H_2$CN); 55.1, 55.0 (2*q*, MeO); 43.2, 43.1, 43.0, 42.9 (Me$_2$*C*HN); 30.6, 30.1 (2*t*, C(2'), C(3')); 24.6, 24.5, 24.4, 24.3, 24.2, 24.1 (*Me*$_2$CHN); 22.4 (*dt*, C(2'), C(3')); 20.4, 20.1 (2*dt*, $J_P$ = 7.2, OCH$_2$*C*H$_2$CN).

$^{31}$P-NMR (121 MHz, $CDCl_3$): 149.2, 148.5.

ESI-MS: 767.4 (100, $[m+Na]^+$); 303.2 (12).

**$N^4$-benzoyl-1-{4'-O-[(cyanoethoxy)(diisopropylamino)phosphino]-2',3'-dideoxy-6'-O-[(4,4'-dimethoxytriphenyl)methyl]-$\beta$-D-glucopyranosyl}cytosine (66)**




After drying for 4.5 h under high vacuum ($p < 10^{-1}$ mbar) at rt, 278 mg (0.430 mmol) **62** was disolved under Ar at rt in 11 ml dry THF and 0.22 ml (1.26 mmol) (iPr)$_2$EtN (bp 128° C, distilled over $CaH_2$) and 0.14 ml (0.608 mmol) chloro(2-cyanoethoxy)(diisopropylamino)phosphine was dropwisely added. After stirring for 75 min at rt, the reaction was evaporated under reduced pressure ($t$ = 28° C) and dried for 1h under high vacuum. CC (10 g $SiO_2$, $CH_2Cl_2$/acetone/$NEt_3$ 4:1:0.01) and reprecipitation from rapidly stirred $CH_2Cl_2$ (1 ml) with hexane (30 ml) resulted in

305 mg (0.360 mmol, 84%) **66** as a colourless foam in a 1:1-diastereomeric mixture ($^{1}$H- and $^{31}$P-NMR).

$R_f$ ($SiO_2$; EtOAc) 0.37, 0.32 ($UV_{254}$, $Ce(SO_4)_2$).

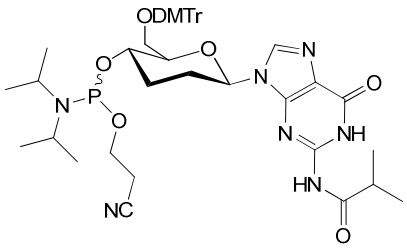
$^{1}$H-NMR (300 MHz, $CD_2Cl_2$): 8.90 (br. *s*, 1H, NH); 8.04 (*d*, *J* = 7.5, 0.5 H, HC(6)); 8.00 (*d*, *J* = 7.5, 0.5 H, HC(6)); 7.95 (*d*, *J* = 7.3, 2 arom. H); 7.64-7.46 (*m*, 6H, HC(5), arom.); 7.41-7.19, 6.88-6.78 (2*m*, 11 arom. H); 5.82 (*dd*, *J* = 10.1, 1.7, HC(1')); 3.88-3.62 (*m*, 3H, HC(4'), OC*H*$_2$CH$_2$CN); 3.78, 3.77 (2*s*, 6H, MeO); 3.50-3.26 (*m*, 5H, HC(5'), HC(6'), Me$_2$C*H*N); 2.62-2.55 (*m*, 1H, OCH$_2$C*H*$_2$CN); 2.41-2.21 (*m*, 3H, HC(2'), HC(3'), OCH$_2$C*H*$_2$CN); 1.88-1.72 (*m*, 1H, HC(2'), HC(3')); 1.58-1.48 (*m*, 1H, HC(2'), HC(3')); 1.17-0.91 (*m*, 12H, *Me*$_2$CHN).

$^{13}$C-NMR (75 MHz, $CD_2Cl_2$): 162.6, 162.5 (2*s*, C(4)); 158.9, 145.5, 145.4 (3*s*, arom. C); 144.5 (*d*, C(6)); 136.4, 136.3, 133.7 (3*s*, 3 arom. C); 133.3, 130.6, 130.5, 130.4, 129.2, 128.7, 128.5, 128.0, 127.1, 127.0, 113.3 (11*d*, arom. C); 118.2, 118.1 (2*s*, CN); 97.1 (*d*, C(5)); 86.1, 86.0 (2*s*, *C*OC(6')); 83.6 (*d*, C(1')); 82.1, 82.0 (2*d*, C(5')); 68.2 (*dd*, $J_P$ = 12.8, C(4')); 67.5 (*dd*, $J_P$ = 16.6, C(4')); 63.9, 63.7 (2*t*, C(6')); 58.6 (*dt*, $J_P$ = 18.9, O*C*H$_2$CH$_2$CN); 58.1 (*dt*, $J_P$ = 20.5, O*C*H$_2$CH$_2$CN); 55.6, 55.5 (2*q*, MeO); 43.4 (*dd*, $J_P$ = 12.2, Me$_2$*C*HN); 43.3 (*dd*, $J_P$ = 12.5, Me$_2$*C*HN); 31.1, 30.9, 30.7, 30.6 (4*t*, C(2'), C(3')); 24.8, 24.7, 24.5, 24.3 (*Me*$_2$CHN); 20.7 (*dt*, $J_P$ = 6.9, OCH$_2$*C*H$_2$CN); 20.5 (*dt*, $J_P$ = 7.1, OCH$_2$*C*H$_2$CN).

$^{31}$P-NMR (121 MHz, $CD_2Cl_2$): 148.8, 148.3.

ESI-MS: 870.5 (100, $[m+Na]^+$); 303.1 (7).

**9-{4'-O-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-2',3'-dideoxy-6'-*O*-[(4,4'-dimethoxytriphenyl)methyl]-$\beta$-D-glucopyranosyl}-$N^2$-Isobutyrylguanine (68)**




To a solution of 150 mg (0.229 mmol) **64** (dried for 13 h under high vacuum ($p < 10^{-1}$ mbar)) and 0.12 ml (0.689 mmol) $(iPr)_2EtN$ (bp 128°C, distilled over $CaH_2$) in 2.2 ml dry THF was added 0.08 ml (0.343 mmol) chloro(2-cyanoethoxy)(diisopropylamino)phosphine under Ar dropwise at rt. After stirring for 1 h at rt, during which a precipitate was formed, the reaction was diluted with $CH_2Cl_2$ (60 ml) (clear solution) and extracted with ice-cold $NaHCO_{3\ sat.}$ (3 x 30 ml) followed by $NaCl_{sat.}$ (30 ml). The combined aqueous solutions were extracted with $CH_2Cl_2$ (50 ml) and the combined organic phases were dried over $MgSO_4$ and evaporated. CC (6 g $SiO_2$, $CH_2Cl_2$/acetone/$Et_3N$ 3:1:0.04) and three times precipitation from 1 ml $CH_2Cl_2$ with 30 ml hexane resulted in 129 mg (0.154 mmol, 67%) **68** as a colourless foam in a 1:1-diastereomeric mixture ($^1$H- and $^{31}$P-NMR).

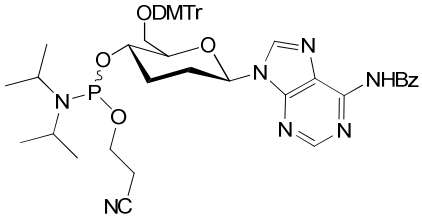
$R_f$ ($SiO_2$; EtOAc) 0.24, 0.17 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, $CD_2Cl_2$): 12.04, 8.90 (br. 2*s*, 2H, NH); 7.92 (*s*, 0.5H, HC(8)); 7.89 (*s*, 0.5H, HC(8)); 7.48-7.45, 7.35-7.30, 7.25-7.16, 6.79-6.73 (4*m*, 13 arom. H); 5.65-5.59 (*m*, 1H, HC(1)); 4.02-3.60 (*m*, 2H, OC*H*$_2$CH$_2$CN); 3.76, 3.75, 3.75, 3.74 (4*s*, 6H, MeO)); 3.52-3.17 (*m*, 6H, HC(4'), HC(5'), HC(6'), $Me_2$C*H*N); 2.61-2.14 (*m*, 5H, HC(2'), HC(3'), $Me_2$C*H*CO, OCH$_2$C*H*$_2$CN); 1.84-1.65 (*m*, 2H, HC(2'), HC(3')); 1.22-0.83 (*m*, 12H, *Me*$_2$CHN, *Me*$_2$CHCO).

$^{13}$C-NMR (75 MHz, $CD_2Cl_2$): 179.4 (*s*, CO); 155.9 (*s*, C(6)); 148.4, 148.3, 147.88, 147.87 (4*s*, C(2), C(4)); 158.9, 145.6, 145.4, 136.4, 136.3 (5*s*, arom. C); 137.0, 136.9 (2*d*, C(8)); 130.6, 130.50, 130.47, 128.7, 128.5, 127.9, 127.0, 126.9, 113.2, 113.1 (10*d*, arom. C); 121.6 (*s*, C(5)); 118.3, 118.1 (2*s*, CN); 86.1 (*s*, *C*OC(6')); 82.2, 82.1 (2*d*, C(1')); 81.6 (*dd*, $J_P$ = 5.3, C(5')); 81.5 (*dd*, $J_P$ = 8.1, C(5')); 68.1 (*dd*, $J_P$ = 13.0, C(4')); 67.5 (*dd*, $J_P$ = 15.4, C(4')); 58.4 (*dt*, $J_P$ = 19.7, O*C*H$_2$CH$_2$CN); 58.0 (*dt*, $J_P$ = 20.9, OCH$_2$CH$_2$CN); 55.5, 55.4 (2*q*, MeO); 43.4 (*dd*, $J_P$ = 5.6, Me$_2$*C*HN); 43.3 (*dd*, $J_P$ = 4.8, Me$_2$*C*HN); 36.6 (*d*, Me$_2$*C*HCO); 31.2, 30.9, 30.8, 30.7 (4*t*, C(2'), C(3')); 24.8, 24.7, 24.6, 24.5, 24.4 (*Me*$_2$CHN); 20.8 (*dt*, $J_P$ = 7.6, OCH$_2$*C*H$_2$CN); 20.6 (*dt*, $J_P$ = 7.4, OCH$_2$*C*H$_2$CN); 19.0 (*q*, *Me*$_2$CHCO).

$^{31}$P-NMR (121 MHz, $CD_2Cl_2$): 148.9, 147.9.

**$N^6$-Benzoyl-9-{4'-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-2',3'-dideoxy-6'-*O*-[(4,4'-dimethoxytriphenyl)methyl]-$\beta$-D-glucopyranosyl]}adenine (67)**




To a solution of 193 mg (0.288 mmol) **63** (dried for 14 h under high vacuum ($p < 10^{-1}$ mbar)), 0.14 ml (0.804 mmol) dry (iPr)$_2$EtN (bp 128°C, distilled from $CaH_2$) in 1.9 ml dry THF was added 0.10 ml (0.434 mmol) chloro(2-cyanoethoxy)(diisopropylamino)phosphine dropwise at rt under Ar. During the addition of the phosphine the yellow solution turned colourless. After stirring for 1 h at rt, during which a precipitate was formed, the reaction was filtered under $N_2$ and the filtrate was evaporated ($t$ = 29° C). The white foaming residue was dissolved in EtOAc (50 ml) and extracted with $NaHCO_{3\ sat}$. (2 x 25 ml), the combined aqueous phases were extracted with EtOAc (30 ml)

and the combined organic phases were dried over $MgSO_4$ and evaporated. CC (8 g $SiO_2$, EtOAc/$CH_2Cl_2$/$Et_3N$ 9:9:2, at $t$ = 8° C) and reprecipitation from 1 ml EtOAc with 30 ml hexane resulted in 196 mg (0.225 mmol, 78%) **67** as colourless foam of a 1:1-diastereomeric mixture ($^1$H- and $^{31}$P-NMR).

$R_f$ ($SiO_2$; EtOAc) 0.39, 0.33 ($UV_{254}$, $Ce(SO_4)_2$).

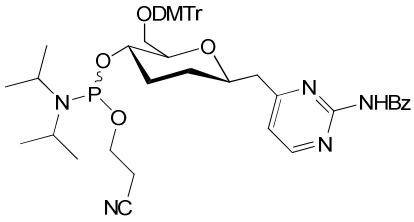
$^1$H-NMR (300 MHz, $CD_2Cl_2$): 9.31 (br. *s*, 1H, NH); 8.76, 8.76 (2*s*, 1H, HC(2)); 8.32, 8.30 (2*s*, 1H, HC(8)); 8.04 (*d*, *J* = 7.0, 2 arom. H); 7.65-7.58, 7.56-7.51, 7.50-7.43, 7.38-7.30, 7.28-7.15, 6.82-6.74 (6*m*, 16 arom. H); 5.98 (*dd*, *J* = 10.5, 2.4, 1H, HC(1')); 4.03-3.62 (*m*, 3H, HC(4'), OC*H*$_2$CH$_2$CN); 3.77, 3.76, 3.76, 3.75 (4*s*, 6H, MeO); 3.56-3.22 (*m*, 5H, HC(5'), HC(6'), Me$_2$C*H*N); 2.62 (*t*, *J* = 6.1, 1H, OCH$_2$C*H*$_2$CN); 2.36 (*t*, *J* = 6.2, 1H, OCH$_2$C*H*$_2$CN); 2.50-2.43, 2.38-2.15, 1.95-1.80 (3*m*, 4H, HC(2'), HC(3')); 1.19-1.08 (*m*, 9H, *Me*$_2$CHN); 0.95 (*d*, *J* = 6.7, 3H, *Me*$_2$CHN).

$^{13}$C-NMR (75 MHz, $CD_2Cl_2$): 169.0 (*s*, CO); 163.0, 162.9 (2*s*, arom. C); 152.7 (*d*, C(2)); 150.6 (*s*, C(4)); 149.7, 149.6, 147.9 (3*s*, arom. C.); 143.4, 143.3 (2*d*, C(8)); 139.0, 138.9, 134.3, 134.2 (4*s*, arom. C); 132.2, 130.7, 128.5, 128.4, 128.4, 128.3, 127.0, 126.6, 126.4, 126.0, 125.8, 124.9, 124.8 (13*d*, arom. C); 121.6 (*s*, C(5)); 116.1, 116.0 (2*s*, CN); 111.2, 111.1 (2*d*, arom. C); 84.1, 84.0 (2*s*, *C*OC(6')); 80.0, 79.9 (2*d*, C(1')); 79.6 (*dd*, $J_P$ = 5.9, C(5')); 79.7 (*dd*, $J_P$ = 8.4, C(5')); 66.2 (*dd*, $J_P$ = 13.4, C(4')); 65.4 (*dd*, $J_P$ = 16.5, C(4')); 61.8, 61.8 (2*t*, C(2')); 56.5 (*dt*, $J_P$ = 19.0, OCH$_2$CH$_2$CN); 56.5 (*dt*, $J_P$ = 20.2, OCH$_2$CH$_2$CN); 53.4, 53.3 (2*q*, MeO); 41.4 (*dd*, $J_P$ = 4.2, Me$_2$*C*HN); 41.2 (*dd*, $J_P$ = 4.2, Me$_2$*C*HN); 29.1, 29.0, 28.9, 28.8 (4*t*, C(2'), C(3')); 22.7, 22.6, 22.5, 22.5, 22.4, 22.3, 20.9, 19.0 (*Me*$_2$CHN); 18.6, 18.4 (2*dt*, $J_P$ = 7.2, OCH$_2$*C*H$_2$CN).

$^{31}$P-NMR (121 MHz, $CD_2Cl_2$): 149.0, 148.4.

ESI-MS: 894.6 (100, $[M+Na]^+$).

**2-benzoylamino-4-({2',3'-dideoxy-4'-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]-6'-*O*-[(4,4'-dimethoxytriphenyl)methyl]-$\beta$-D-glucopyranosyl}methyl)-pyrimidine (77)**




To a solution of 243 mg (0.376 mmol) **75** (dried for 14 h under high vacuum ($p < 10^{-1}$ mbar)) and 0.25 ml (1.44 mmol) $(iPr)_2EtN$ (bp 128° C, distilled over $CaH_2$) in 1.8 ml dry $CH_2Cl_2$ was added at rt under Ar 0.13 ml (0.564 mmol) chloro(2-cyanoethoxy)(diisopropylamino)phosphine dropwise. After stirring for 1 h at rt, the reaction was evaporated ($t$ = 29°C). The yellow foaming residue was dissolved in EtOAc (120 ml) and extracted with ice-cold $NaHCO_{3\ sat.}$ (2 x 50 ml) and $NaCl_{sat.}$ (50 ml). The combined aqueous phases were extracted with EtOAc (40 ml) and the combined organic phases were dried over $MgSO_4$ and evaporated. CC (8 g $SiO_2$, EtOAc/1% $NEt_3$) and reprecipitation from 1 ml $CH_2Cl_2$ with 30 ml hexane resulted in 220 mg (0.260 mmol, 69%) **77** as a slightly yellow foam of a 1:1-diastereomeric mixture ($^1$H- and $^{31}$P-NMR).

$R_f$ ($SiO_2$; EtOAc) 0.39, 0.33 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, $CD_2Cl_2$): 8.69-8.63 (*m*, 1H); 8.58 (br. *s*, 1H, NH); 8.49 (*t*, *J* = 4.8, 1H); 7.95-7.87 (*m*, 1H); 7.75-7.68 (*m*, 3H); 7.61-7.12 (*m*, 24H, arom. H); 6.86-6.77 (*m*, 7H); 3.95-3.85 (*m*, 1H); 3.78, 3.77, 3.76, 3.75 (4*s*, 12H, MeO); 3.72-2.83 (*m*, 16H); 2.66-2.57 (*m*, 1H); 2.55 (*t*, *J* = 6.3, 1H); 2.36-2.19 (*m*, 3H); 1.90-1.72, 1.70-1.51 (2*m*, 4H); 1.22-1.03 (*m*, 17H); 1.12, 1.07, 0.87 (3*d*, *J* = 6.8, 19H).

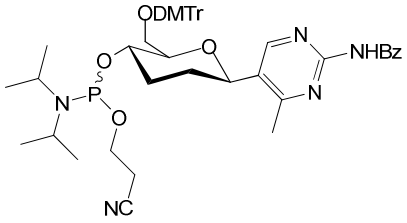
$^{13}$C-NMR (75 MHz, $CD_2Cl_2$): 170.3, 170.2, 165.0, 164.9, 158.9, 158.8, 158.7 (7*s*, CO, arom. C); 158.2 (*d*, arom. C); 145.8, 145.7 (2*s*, arom. C); 136.8, 136.7, 136.6, 135.1, 135.0 (5*s*, arom. C); 132.4, 132.3, 130.5, 130.46, 130.43, 130.3, 129.0, 128.9, 128.7, 128.5, 128.0, 127.7, 127.6, 126.9, 126.8 (15*d*, arom. C); 118.1, 118.0 (2*s*, CN); 117.20, 117.16 (2*d*, NCH*C*H$_{arom.}$); 113.3, 113.2 (2*d*, arom. C); 85.91, 85.89 (2*s*, *C*OC(6')); 81.4 (*dd*, $J_P$ = 6.0, C(5')); 81.3 (*dd*, $J_P$ = 8.1, C(5')); 76.5 (*d*, C(1')); 69.9 (*dd*, $J_P$ = 13.5, C(4')); 68.9 (*dd*, $J_P$ = 15.2, C(4')); 64.4, 64.2 (*t*, C(6')); 58.5 (*dt*, $J_P$ = 18.6, O*C*H$_2$CH$_2$CN); 58.1 (*dt*, $J_P$ = 19.7, O*C*H$_2$CH$_2$CN); 55.5, 55.4 (2*q*, MeO); 44.2 (*t*, C(1')*C*H$_2$-arom.); 43.4 (*dd*, $J_P$ = 3.3, Me$_2$*C*HN); 43.3 (*dd*, $J_P$ = 3.4, Me$_2$*C*HN); 32.4, 32.2, 31.4, 31.3 (4*t*, C(2'), C(3')); 24.7, 24.6, 24.5, 24.4, 24.3 (*Me*$_2$CHN); 20.7 (*dt*, $J_P$ = 7.1, OCH$_2$*C*H$_2$CN); 20.5 (*dt*, $J_P$ = 7.1, OCH$_2$*C*H$_2$CN).

$^{31}$P-NMR (121 MHz, $CD_2Cl_2$): 148.1, 147.8.

ESI-MS: 868.7 (100, [m+Na]$^+$), 303.2 (5).

HR-ESI-MS: calc. for $C_{48}H_{56}N_5OPNa$ ([m+Na]$^+$) 868.3815; found 868.3808.

**2-aminobenzoyl-4-methyl-5-{2',3'-dideoxy-4'-*O*-[(2-cyanoethoxy)(diisopropylamino)phosphino]6'-*O*-[(4,4'-dimethoxytriphenyl)methyl]-*β*-D-glucopyranosyl}-pyrimidine (78)**



After drying for 14 h under high vacuum ($p < 10^{-1}$ mbar) 342 mg (0.530 mmol) **76** dissolved under Ar at rt in 13 ml dry THF and 0.30 ml (1.72 mmol) $(iPr)_2EtN$ (bp 128° C, distilled over $CaH_2$) and 0.17 ml (0.794 mmol) chloro(2-cyanoethoxy)(diisopropylamino)phosphine was dropwisely added. After stirring for 70 min at rt, the reaction was evaporated ($t$ = 29°C). CC (10 g $SiO_2$, EtOAc/0.2%$NEt_3$) and reprecipitation from 1 ml $CH_2Cl_2$ with 30 ml hexane resulted in 251 mg (0.297 mmol, 56%) **78** as slightly yellow foam of a 1:1-diastereomeric mixture ($^1$H- and $^{31}$P-NMR).

$R_f$ ($SiO_2$; EtOAc) 0.62, 0.55 ($UV_{254}$, $Ce(SO_4)_2$).

$^1$H-NMR (300 MHz, $CD_2Cl_2$): 9.10 (br. *s*, NH); 8.61, 8.58 (2*s*, HC(6)); 7.92-7.89, 7.81-7.78, 7.55-7.18, 6.83-6.79 (4*m*); 4.61 (*dd*, *J* = 8.1, 1.9); 3.92-3.62 (*m*); 3.77, 3.763, 3.758, 3.756 (4*s*, MeO); 3.59-3.05 (*m*); 2.58, 2.57 (2*s*, $H_3C_{arom}$); 2.01-1.98, 1.83-1.73, 1.65-1.56 (3*m*, $H_2C$(2'), $H_2C$(3')); 1.27-0.85 (*m*).

$^{13}$C-NMR (75 MHz, $CD_2Cl_2$): 166.9, 166.8 (2*s*); 165.4 (*d*); 158.8, 157.0, 155.7, 145.7, 145.6, 136.7, 136.5 (7*s*); 135.2, 132.5, 130.64, 130.59, 130.55, 130.5, 129.3, 129.2, 129.0, 128.9, 128.8, 128.5, 128.0, 127.9, 127.5, 127.0, 126.9 (17*d*, arom. C); 118.3, 118.1 (2*s*, CN); 113.3, 113.2 (2*d*); 86.14, 86.08 (2*s*, *C*OC(6')); 82.33, 82.28 (2*d*); 74.7. 74.6 (2*d*, C(1')); 69.3 (*dd*, $J_P$ = 13.4, C(4')); 68.4 (*dd*, $J_P$ = 16.6, C(4')); 64.6, 64.4 (2*t*, C(6')); 58.6 (*dt*, $J_P$ = 18.9, O*C*$H_2$$CH_2$CN); 58.2 (*dt*, $J_P$ = 19.3, O*C*$H_2$$CH_2$CN); 55.5 (*q*, MeO); 43.5, 43.3 (2*d*, $Me_2$*C*HN); 32.6, 32.4, 31.0 (3*t*, C(2'), C(3')); 24.8, 24.7, 22.1 (3*q*, *Me*$_2$CHN); 20.7, 20.5 (2*t*, $OCH_2$*C*$H_2$CN).

$^{31}$P-NMR (121 MHz, $CD_2Cl_2$): 148.2, 147.0.

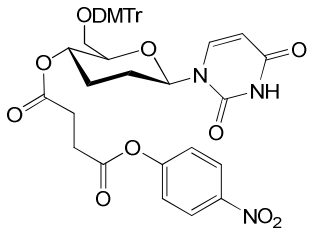
ESI-MS: 884.4 (14, [m+K]), 868.6 (100, $[m+Na]^+$), 846.5 (21, [m+H]).

HR-ESI-MS: calc. for $C_{48}H_{56}N_5OPNa$ ($[m+Na]^+$) 868.3815; found 868.3812.

*Common procedure for the synthesis of the 4-nitrophenylesters **69-72***

To a 0.1- 0.5 M solution of the dimetoxytrityl derivative **61-64** in pyridine was added 1.0-1.2 equiv. 4-(dimethylamino)pyridine and 1.1-1.7 equiv. succinic anhydride. The mixture was stirred for 16-20 h at rt, evaporated and the remaining pyridine was coevaporated with toluene. The crude product was dissolved in $CH_2Cl_2$ and extracted with a 10% aq. citric acid solution. The aqueous phase was extracted with $CH_2Cl_2$ and the combined organic phases were dried ($MgSO_4$), evaporated and dried for several hours at rt under high vacuum ($p < 10^{-1}$ mbar). After proving the formation of the succinic monoesters by ESI-MS, the monoesters were dissolved in dioxane/pyridine 10:1 to 20:1 (concentration 0.15-0.2 M) without further purification, 1.0-1.7 equiv. 4-nitrophenol and 1.9-2.5 equiv. DCC was added and the reaction was stirred for 3-4 h at rt. The formed *N*,*N*'-dicyclohexylurea was filtered off, washed with dioxane, and the filtrate was evaporated. Remaining traces of pyridine were removed by coevaporation with toluene. Purification by CC ($SiO_2$) followed by evaporation and drying at rt with high vacuum resulted in colourless to slightly yellow foams **69-72**.

**1-{2',3'-dideoxy-6'-*O*-[(4,4'-dimethoxytriphenyl)methyl]-4'-*O*-[4-*O*-(4-nitrophenyl)succinyl]-*β*-D-glucopyranosyl}uracil (69)**




Dissolving 892 mg (1.64 mmol) of **61**, 220 mg (1.80 mmol) DMAP and 229 mg (2.29 mmol) succinic anhydride in 5.5 ml pyridine resulted in 1.03 g (1.60 mmol, 98 %) of monoester

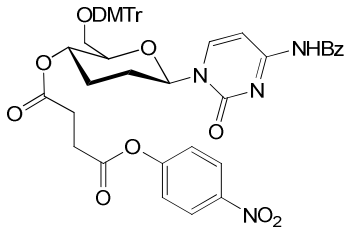
according to common procedure, from which 300 mg (0.465 mmol) were converted to **69** with 91 mg (0.651 mmol) 4-nitrophenol and 211 mg (1.023 mmol) DCC in 2.8 ml dioxane/pyridine 15:1. CC (30 g $SiO_2$, EtOAc/hexane 7:3) resulted in 280 mg (0.365 mmol, 79%) of **69**.

$R_f$ ($SiO_2$; EtOAc) 0.46 (heat (yellow, hydrolysis of p-$NO_2$-phenylester); $Ce(SO_4)_2$ (red, cleavage of the DMTr-group) followed by heat (brown); $UV_{254}$).

$^1$H-NMR (300 MHz, d$^6$-DMSO): 8.27 (*d*, *J* = 9.1, 2 arom. H); 7.80 (*d*, *J* = 8.0, HC(6)); 7.39-7.34, 7.30-7.17, 6.86-6.81 (3*m*, 15 arom. H); 5.75-5.71 (*m*, 2H, HC(1'), HC(5)); 4.96-4.86 (*m*, HC(4')); 3.89-3.84 (*m*, HC(5')); 3.72 (*s*, 2MeO); 3.14-3.09 (*m*, 1HC(6')); 2.94 (*dd*, *J* = 10.2, 4.1, 1HC(6')); 2.78-2.74 (*m*, 1H of OCC$H_2$C$H_2$CO); 2.67-2.35 (*m*, 3H of OCC$H_2$C$H_2$CO); 2.35-0.98 (*m*, $H_2$C(2'), $H_2$C(3')).

ESI-MS: 788.4 (100, $[m+Na]^+$).

**$N^4$-benzoyl-1-{2',3'-dideoxy-6'-*O*-[(4,4'-dimethoxytriphenyl)methyl]-4'-*O*-[4-*O*-(4-nitrophenyl)succinyl]-$\beta$-D-glucopyranosyl}cytosine (70)**




200 mg (0.309 mmol) of **62**, 41 mg (0.336 mmol) DMAP and 43 mg (0.430 mmol) succinic anhydride in 2.0 ml pyridine resulted, according to common procedures, in 220 mg (0.294 mmol, 95 %) of monoester, from which 220 mg (0.294 mmol) were converted to **70** with 57 mg (0.410

mmol) 4-nitrophenol and 134 mg (0.649 mmol) DCC in 1.6 ml dioxane/pyridine 15:1. CC (15 g $SiO_2$, EtOAc/hexane 3:1) resulted in 229 mg (0.264 mmol, 90 %) of **70**.

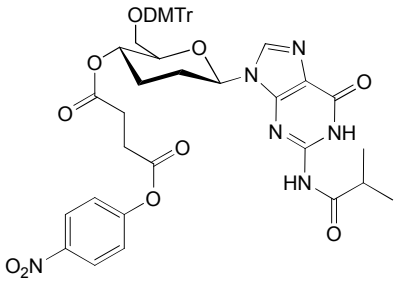
$R_f$ ($SiO_2$; EtOAc) 0.27 (heat (yellow, hydrolysis of p-$NO_2$-phenylester); $Ce(SO_4)_2$ (red, cleavage of the DMTr-group) followed by heat (brown); $UV_{254}$).

$^1$H-NMR (300 MHz, $CD_2Cl_2$): 8.92 (br. *s*, NH); 8.23-8.19 (*m*, 2 arom. H); 7.98-7.88 (*m*, 3H, 2 arom. H, HC(6)); 7.59 (*tt*, *J* =7.3, 1.4, 1 arom. H); 7.52-7.47, 7.42-7.39, 7.29-7.18, 6.81-6.76 (3*m*, HC(5), 17 arom. H); 5.79 (*dd*, *J* = 10.0, 1.9, HC(1')); 5.05-4.95 (*m*, HC(4')); 3.82-3.77 (*m*, HC(5')); 3.73, 3.72 (2*s*, 2MeO); 3.29 (*dd*, *J* = 10.5, 1.8, 1H of $H_2C$(6')); 3.13 (*dd*, *J* = 10.6, 4.3, 1H of $H_2C$(6')); 2.75-2.70 (*m*, 2H of OCC$H_2$C$H_2$CO); 2.61-2.40 (*m*, 2H of OCC$H_2$C$H_2$CO); 2.32-2.29 (*m*, 2H of $H_2C$(2'), $H_2C$(3')); 1.85-1.61 (*m*, 2H of $H_2C$(2'), $H_2C$(3')).

ESI-MS: 891.5 (100, $[m+Na]^+$).

HR-ESI-MS: calc. for $C_{48}H_{44}N_4O_{12}Na$ ($[m+Na]^+$) 891.2853; found 891.2859.

**9-{2',3'-dideoxy-6'-*O*-[(4,4'-dimethoxytriphenyl)methyl]-4'-*O*-[4-*O*-(4-nitrophenyl)succinyl]-$\beta$-D-glucopyranosyl}-$N^2$-isobutyrylguanine (72)**

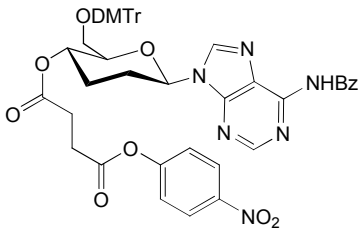


25 mg (38.2 μmol) of **64**, 5.2 mg (42.6 μmol) DMAP and 5.4 mg (54.0 μmol) succinic anhydride in 0.6 ml pyridine resulted, according to common procedures, in 25 mg (33.2 μmol, 87 %) of monoester, from which 25 mg (33.2 μmol) were converted to **72** with 5.2 mg (37.4 μmol) 4-nitrophenol and 12 mg (58.2 μmol) DCC in 0.4 ml dioxane/pyridine 15:1. CC (4 g $SiO_2$, EtOAc/MeOH 40:1) resulted in 21 mg ( 24.0 μmol, 72 %) of **72**.

$R_f$ ($SiO_2$; EtOAc/MeOH 40:1) 0.33 (heat (yellow, hydrolysis of p-$NO_2$-phenylester); $Ce(SO_4)_2$ (red, cleavage of the DMTr-group) followed by heat (brown); $UV_{254}$).

ESI-MS: 897.6 (100, $[m+Na]^+$).

**$N^6$-Benzoyl-9-{2',3'-dideoxy-6'-*O*-[(4,4'-dimethoxytriphenyl)methyl]-4'-*O*-[4-*O*-(4-nitrophenyl)succinyl]-β-D-glucopyranosyl}adenine (71)**




40 mg (59.5 μmol) of **63**, 8.3 mg (67.9 μmol) DMAP and 5.4 mg (85.9 μmol) succinic anhydride in 1.0 ml pyridine resulted, according to common procedures, in 42 mg (54.5 μmol, 92 %) of monoester, from which 42 mg (54.5 μmol) were converted to **71** with 11 mg (79.1 μmol) 4-nitrophenol and 28 mg (0.136 mmol) DCC in 0.6 ml dioxane/pyridine 15:1. CC (4 g $SiO_2$, EtOAc/MeOH 20:1, 1% $NEt_3$) resulted in 40 mg (44.8 μmol, 82 %) **71**.

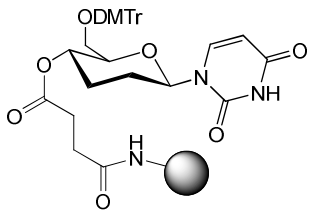
$R_f$ ($SiO_2$; EtOAc/hexane 3:1) 0.25 (heat (yellow, hydrolysis of p-$NO_2$-phenylester); $Ce(SO_4)_2$ (red, cleavage of the DMTr-group) followed by heat (brown); $UV_{254}$).

ESI-MS: 915.4 (100, $[m+Na]^+$).

*Common procedure for preparation of nucleoside-bonded "controlled pore glass" (CPG)*

To a suspension of long-chain alkylamine CPG (*SynGen*, pore diameter 500 Å, mesh size 175-125 μm, substitution 60-80 μmol/g or *3-prime*, pore diameter 500 Å, mesh size 75-125 μm, substitution 85-160 μmol/g) in DMF/$Et_3N$ 10:1 was added a solution of the nitrophenylesters **69-72** in dioxane and the suspension was allowed to stand under swirling for 20-30 h. Immediately after the addition of the activated esters, the suspension turned yellow, due to the formation of 4-nitrophenol. Filtration over a G3 glass filter funnel (max. pore size 16-40 μm); followed by washing with DMF, MeOH and $Et_2O$ (3 x 10 ml) and drying of the resin under high vacuum ($p < 10^{-1}$ mbar) at rt; the loading was determined according to the following procedure: an exactly defined amount of derivatized LCAA-CPG-resin (0.500-2.000 mg) was suspended in a defined volume of 0.1 M TsOH in MeCN or 2% dichloroacetic acid in $CH_2Cl_2$ (10.0 ml). From the supernatant solution, the absorption at 498 nm was determined. From the concentration of the (4,4'-dimethoytriphenyl)methyl-cation ($\varepsilon_{498}$ = 70000) the loading of the CPG-resin [μmol/g] was calculated. If the loading was > 10 μmol/g, the remaining free amino groups were capped with $Ac_2O$, 4-(dimethylamino)pyridine in pyridine for 20 min. This process was repeated until a ninhydrin test (A few beads of derivatised LCAA-CPG one drop of a solution of 2 ml 0.1 M $KCN_{aq}$ in 100 ml pyridine, one drop of a solution of 80 g phenol in 20 ml EtOH and one drop of a solution of 500 mg ninhydrine in 10 ml EtOH were heated for 5 min at 110° C (positive: violet, negative: red-brown))[81] showed no remaining free amino groups on the LCAA-CPG.

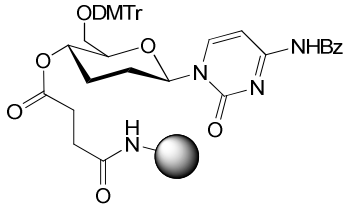
**homo-DNA-U-LCAA-CPG**




Treating 200 mg LCAA-CPG (*3-prime*) in 0.44 ml DMF/$NEt_3$ 10:1 with 12 mg (15.7 μmol) p-nitroester **69** in 0.15 ml dioxane, according to common procedures, followed by capping with 0.12 ml (130 mg, 1.27 mmol) $Ac_2O$ and 7 mg (57.3 μmol) DMAP in 0.6 ml pyridine resulted in a derivatized homo-DNA-U-LCAA-CPG with a loading of 44.5 μmol/g.

Treating 198 mg LCAA-CPG (*SynGen*) in 0.66 ml DMF/$NEt_3$ 10:1 with 12 mg (15.7 μmol) p-nitroester **69** in 0.15 ml dioxane, according to common procedures, followed by capping with 0.12 ml (130 mg, 1.27 mmol) $Ac_2O$ and 7 mg (57.3 μmol) DMAP in 0.6 ml pyridine resulted in a derivatized homo-DNA-U-LCAA-CPG with a loading of 35.0 μmol/g.

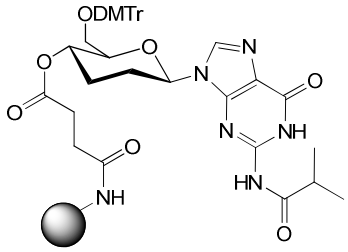
**homo-DNA-C(*N*Bz)-LCAA-CPG**




Treating 360 mg LCAA-CPG (*SynGen*) in 0.99 ml DMF/$NEt_3$ 10:1 with 25 mg (28.8 μmol) p-nitroester **70** in 0.32 ml dioxane, according to common procedures, followed by capping with 0.22

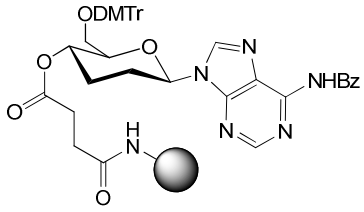
ml (239 mg, 2.34 mmol) $Ac_2O$ and 13 mg (0.106 mmol) DMAP in 1.1 ml pyridine resulted in a derivatized homo-DNA-C(NBz)-LCAA-CPG with a loading of 41.8 μmol/g.

**homo-DNA-G(*N*Ibz)-LCAA-CPG**




Treating 240 mg LCAA-CPG (*SynGen*) in 0.66 ml DMF/$NEt_3$ 10:1 with 17 mg (19.4 μmol) p-nitroester **72** in 0.32 ml dioxane, according to common procedures, followed by capping with 0.22 ml (239 mg, 2.34 mmol) $Ac_2O$ and 13 mg (0.106 mmol) DMAP in 1.1 ml pyridine resulted in a derivatized homo-DNA-G(NIbz)-LCAA-CPG with a loading of 13.9 μmol/g.

**homo-DNA-A(*N*Bz)-LCAA-CPG**




Treating 240 mg LCAA-CPG (*SynGen*) in 0.66 ml DMF/$NEt_3$ 10:1 with 16 mg (17.9 μmol) p-nitroester **71** in 0.32 ml dioxane, according to common procedures, followed by capping with 0.15 ml (163 mg, 1.60 mmol) $Ac_2O$ and 9 mg (73.7 μmol) DMAP in 0.7 ml pyridine resulted in a derivatized A(NBz)-LCAA-CPG with a loading of 44.2 μmol/g.

*Common procedure for the manual synthesis of oligonucleotides with cyanoethyl-phosphoramidites (methode I)*

1.) *Detritylation*: Nucleoside-loaded CPG-solid support was suspended in a G3 glass filter funnel with 3 ml of 2% dichloroacetic acid in $CH_2Cl_2$, shaken and the 2% dichloroacetic acid was filtered off by applying a slight over pressure with $N_2$. The same procedure was repeated twice over 2 min. The filtrates were collected and the concentration of $(MeO)_2$Tr-cation was determined. The solid support was immediately washed with $CH_2Cl_2$ (3 x 5 ml) and $Et_2O$ (3 x 5 ml) and dried by flowing $N_2$ through the sample.

2.) *Coupling*: The solid support was dried together with 8 eq of solid phosphoramidite and 30 eq of solid 5-(ethylthio)-1*H*-tetrazole at rt over 45 min, flushed with Ar and suspended with dry $CH_3CN$ (0.2 ml per 20 mg phosphoramidite). After 1 h reaction time, under careful rotation, the solution was filtered off and the solid support was washed with dry $CH_3CN$ (3 x 5 ml), dry THF (3 x 5 ml) and dried by flowing $N_2$ through the sample for 5 min.

3.) *Capping*: The solid support was suspended under Ar with 2 ml of a freshly prepared solution of 0.5 ml capping solution I (6.50 g DMAP / 100 ml dry THF) and 1.5 ml of capping solution II (10 ml $Ac_2O$ / 15 ml 2,6-dimethylpyridine). After 5 min reaction time, the solution was filtered off and the solid support was washed with dry THF (3 x 5 ml), MeOH (3 x 2 ml), THF/$H_2O$/2,6-dimethylpyridine 2:2:1 (1 x 2 ml).

4.) *Oxidation*: 3 ml of a 0.1 M $I_2$ solution in THF/$H_2O$/2,6-dimethylpyridine 2:2:1 was added to the solid support. After 1 min reaction time, the solution was filtered off and the resin washed with THF/$H_2O$/2,6-dimethylpyridine 2:2:1 (3 x 2 ml), MeOH (3 x 5 ml), and dry $Et_2O$ (3 x 3 ml) and dried under a permanent flow of $N_2$ for 5 min.

5.) *Deprotection of the phosphotriester-groups and cleavage from the solid support:* The solid support was suspended in 8 ml conc. $NH_{3\ aq}$ for 20 h at 55° C. The solution was

filtered off and the resin was washed with conc. $NH_{3\ aq}$ (3 x 3 ml) and $H_2O$ (3 x 1 ml). The solution was evaporated to dryness, 5 ml $H_2O$ was added and the suspension was sonicated for 2 min, filtered over a *Micropore* (0.45 μm) membrane-filter and lyophilised. In this form the crude oligonucleotide was purified by HPLC.

**ddGlc[UU]**

$t_R$ ($C_{18}$-Waters Spherisorb ODS2, 5 μm, 250 x 4.6 mm; gradient 2 min 100% 0.1 M ($HNEt_3$)OAc pH 7.0, until 15 min 80% 0.1 M ($HNEt_3$)OAc pH 7.0/ 20% $CH_3CN$, during 5 min 80% 0.1 M ($HNEt_3$)OAc pH 7.0/ 20% $CH_3CN$, 2.5 min 0.1 M ($HNEt_3$)OAc pH 7.0): 13.7 min.

ESI-MS (positiv): 569.1 (70, $[M+H+Na]^+$), 547.1 (100, $[M+2H]^+$).

ESI-MS (negative): 545.1 (100, $[M^-]$).

HR-ESI-MS (negative): calc. for $C_{20}H_{26}N_4O_{12}P_1$ ($[M^-]$) 545.1285, found 545.1291.

UV (0.1 M ($HNEt_3$)OAc pH 7.0): 259.

*Automated solid-phase synthesis on a "DNA synthesizer" (Expedite NAS 8905, Applied Biosystems: methode II)*

With exception of the phosphoramidite-solutions (0.1 M or 0.05 M) and the activating solution (5-(ethylthio)-1*H*-tetrazole instead of 1*H*-tetrazole) the original reagents from *Applied Biosystems* were used:

1. *Detritylation*: 3% trichlororacetic acid in $CH_2Cl_2$.

2. *Coupling*: 0.5 M 5-(ethylthio)-1*H*-tetrazole in $CH_3CN$ (*Fluka*, dry, over molecular sieve ($H_2O$ < 10 ppm)), 0.1 M (0.05 M) phosphoramidite solution in $CH_3CN$ (*Fluka*, dry, over molecular sieve ($H_2O$ < 10 ppm)).
3. *Capping*: 7% 1-methylimidazole in THF/2,6-dimethylpyridine/$Ac_2O$ 15:1:1.
4. *Oxidation*: 0.1 M $I_2$ solution in THF/pyridine/$H_2O$.
5. *Wash*: $CH_3CN$ (*Fluka*, dry, over molecular sieve ($H_2O$ < 0.01%)).

For the oligonucleotide synthesis, standard parameters were used with exception of the coupling time, which was in increased for some couplings from 90 s to 3 min, 30 s. In later oligonucleotide synthesis (with 0.05 M phosphoramidite solutions in $CH_3CN$) typically preparations were performed on 0.4 µmol scale according to the 1 µmol protocol (25 eq. phosphoramidites). For all oligonucleotide synthesis on 0.4 µmol scale (with 0.05 M 2-(cyanoethyl)phosphoramidite solutions) 3 Å molecular sieve (dried for 14 h at 95° C under high vacuum ($p < 10^{-1}$ mbar)) was added to all phosphoramidites, to the activator (5-ethylthio-1*H*-tetrazole) and to the wash acetonitril. The phosphoramidite solutions were prepared with acetonitril from *Fluka* (dry, over molecular sieve, $H_2O$ < 10 ppm). For the wash-acetonitrile, dry acetoniril from *Fluka* (over molecular sieve, $H_2O$ < 0.01%) was used. Oligonucleotides were synthesised without exception in the "auto-trityl-off-mode" (automatic cleavage of the of the last $(MeO)_2Tr$-group). After synthesis, the solid support was suspended in conc. $NH_{3\ aq}$ at 55° C for 16-20 h. The solutions were decanted and the resin was washed with conc. $NH_{3\ aq}$ (3 x 0.3 ml) and $H_2O$ (2 x 0.2 ml). From the combined solutions the volatile $NH_3$ was removed in a sonicated water bath (50° C, 30 min). The solutions were lyophilised, dissolved in $H_2O$ and filtered over a *Micropore* (0.20 µm) membrane filter (*Whatman Schleicher & Schuell*, membrane: cellulose acetate). In this form the crude oligomer was used for analysis and purification by HPLC, respectively, if the HPLC showed only a single peak, by quantitative ethanol precipitation: The crude oligonucleotide was dissolved in 300 µl water. 30 µl 3 M Na-acetate-buffer (pH 4.8) was added, followed by 990 µl EtOH and the solution was freezed for at least 12 h at -20° C and spinned for 30 min at 6000 rpm.

The supernatant was decanted and the pellet carefully washed by adding 1 ml ice-cold 70% EtOH in $H_2O$. The supernatant was decanted and the pellet dried under vacuum.

If the analytical HPLC showed more than one peak the crude oligonucleotide was purified by preparative RP-$C_{18}$- ($C_{18}$-Waters Spherisorb ODS2, 5 µm, 250 x 4.6 mm) or DEAE-IE-HPLC (Nucleogen-DEAE 60-7, 7 µm, 125 x 4 mm), followed by removal of the buffer salts by Sep-Pak®-$C_{18}$-Cartridges (*Waters*).

**ddGlc[$U_3$]**

scale: 0.2 µmol

synthesis protocol: 0.2 µmol

coupling time: 3 min 30 s

$t_R$ ($C_{18}$-Waters Spherisorb ODS2, 5 µm, 250 x 4.6 mm; gradient 2 min 100% 0.1 M ($HNEt_3$)OAc pH 7.0, until 25 min 70% 0.1 M ($HNEt_3$)OAc pH 7.0 / 30% $CH_3CN$): 15.2 min.

UV (0.1M ($HNEt_3$)OAc pH 7.0): 260.

**ddGlc[$U_4$]**

scale: 0.2 µmol

synthesis protocol: 0.2 µmol

coupling time: 1 min 30 s

$t_R$ ($C_{18}$-Waters Spherisorb ODS2, 5 µm, 250 x 4.6 mm; gradient 2 min 100% 0.1 M ($HNEt_3$)OAc pH 7.0, until 25 min 70% 0.1 M ($HNEt_3$)OAc pH 7.0 / 30% $CH_3CN$): 15.3 min.

UV (0.1 M ($HNEt_3$)OAc pH 7.0): 260.

**ddGlc[$U_6$]**

scale: 0.2 μmol

synthesis protocol: 0.2 μmol

coupling time: 3 min 30 s

$t_R$ ($C_{18}$-Waters Spherisorb ODS2 5 μm, 250 x 4.6 mm; gradient 2 min 100% 0.1 M ($HNEt_3$)OAc pH 7.0, until 10 min 50% 0.1 M ($HNEt_3$)OAc pH 7.0 / 50% $CH_3CN$): 8.4 min.

UV (0.1 M ($HNEt_3$)OAc pH 7.0): 260.

**ddGlc[$^{4'}$(U-A)$_3{}^{6'}$]**

scale: 0.2 μmol

synthesis protocol: 0.2 μmol

coupling time: 3 min 30 s

$t_R$ ($C_{18}$-Waters Spherisorb ODS2, 5 μm, 250 x 4.6 mm; gradient 2 min 100% 0.1 M ($HNEt_3$)OAc pH 7.0, until 25 min 70% 0.1 M ($HNEt_3$)OAc pH 7.0 / 30% $CH_3CN$): 18.0 min.

ESI-MS (negative) : 936.6 (27, $[M+H+2Na]^{2-}$), 933.6 (34, $[M+2H+K]^{2-}$), 925.6 (56, $[M+2H+Na]^{2-}$), 914.7 (100, $[M+3H]^{2-}$).

UV (0.1 M ($HNEt_3$)OAc pH 7.0): 259.

**ddGlc[$^{4'}$(U-A)$_4{}^{6'}$]**

scale: 0.2 μmol

synthesis protocol: 0.2 μmol

coupling time: 3 min 30 s

$t_R$ ($C_{18}$-Waters Spherisorb ODS2, 5 μm, 250 x 4.6 mm; gradient 2 min 100% 0.1 M ($HNEt_3$)OAc pH 7.0, until 25 min 70% 0.1 M ($HNEt_3$)OAc pH 7.0 / 30% $CH_3CN$): 18.5 min.

ESI-MS (negative) : 1252.6 (100, $[M+3H+Na]^{2-}$), 1241.1 (98, $[M+4H+Na]^{2-}$), 1230.5 (42, $[M+5H]^{2-}$).

UV (0.1 M ($HNEt_3$)OAc pH 7.0): 258.

**ddGlc[$C_7$]**

scale: 0.2 μmol

synthesis protocol: 0.2 μmol

coupling time: 1 min 30 s

$t_R$ ($C_{18}$-Waters Spherisorb ODS2, 5 μm, 250 x 4.6 mm; gradient 2 min 100% 0.1 M ($HNEt_3$)OAc pH 7.0, until 25 min 70% 0.1 M ($HNEt_3$)OAc pH 7.0 / 30% $CH_3CN$): 16.4 min.

MALDI-TOF-MS (Matrix ATT): calc. ddGlc[$C_7$ + 5H]$^-$) 2058.7, found 2058.4.

UV ($H_2O$): 270.

**ddGlc[$G_7$]**

scale: 0.2 μmol

synthesis protocol: 0.2 μmol

coupling time: 3 min 30 s

MALDI-TOF-MS (Matrix ATT): calc. ddGlc[$G_7$ + 5H]$^-$) 2338.5, found 2337.8.

UV ($H_2O$): 252.

**ddGlc[$^{4'}$UAUD(UA)$_2$$^{6'}$]**

scale: 0.4 μmol

synthesis protocol: 1.0 μmol (0.05 M phosphoramidite solution)

coupling time: 1 min 30 s

$t_R$ (*Nucleogen*-DEAE 60-7, 7 μm, 125 x 4 mm, gradient 2 min 85% 20 mM $KH_2PO_4$ $H_2O$/$CH_3CN$ 4:1, pH 6.0, 15% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O$/$CH_3CN$ 4:1, pH 6.0 until 30 min 25% 20 mM $KH_2PO_4$ $H_2O$/$CH_3CN$ 4:1, pH 6.0, 75% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O$/$CH_3CN$ 4:1, pH 6.0): 15.5 min.

UV (20 mM $KH_2PO_4$ in $H_2O$/$CH_3CN$ 4:1, pH 6.0): 258.

MALDI-TOF-MS (Matrix ATT): calc. ddGlc[UAUD(UA)$_2$ + 6H]$^-$) 2435.5, found 2435.5.

**ddGlc[$^{4'}$UD(UA)$_3$$^{6'}$]**

scale: 0.4 μmol

synthesis protocol: 1.0 μmol (0.05 M phosphoramidite)

coupling time: 1 min 30 s

$t_R$ (Nucleogen-DEAE 60-7, 7 µm, 125 x 4 mm, gradient 2 min 85% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 15% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0 until 30 min 25% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 75% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0): 14.6 min.

UV (20 mM $KH_2PO_4$ in $H_2O/CH_3CN$ 4:1, pH 6.0): 258.

MALDI-TOF-MS (Matrix ATT): calc. ddGlc[UD$(UA)_3$ + 6H]$^-$) 2435.5, found 2435.3.

**ddGlc[$^{4'}$$(UA)_2$UD$(UA)_2$$^{6'}$]**

scale: 0.4 µmol

synthesis protocol: 1.0 µmol (0.05 M 2-(cyanoethyl)phosphoramidite)

coupling time: 1 min 30 s

$t_R$ (Nucleogen-DEAE 60-7, 7 µm, 125 x 4 mm, gradient 2 min 85% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 15% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0 until 30 min 25% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 75% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0): 17.6 min.

UV (20 mM $KH_2PO_4$ in $H_2O/CH_3CN$ 4:1, pH 6.0): 258.

MALDI-TOF-MS (Matrix ATT): calc. ddGlc[$(UA)_2$UD$(UA)_2$ + 8H]$^-$) 3066.6, found 3066.5

**ddGlc[$^{4'}$$UD_8$$^{6'}$]**

scale: 0.4 µmol

synthesis protocol: 1.0 µmol (0.05 M 2-(cyanoethyl)phosphoramidite)

coupling time: 1 min 30 s

$t_R$ (Nucleogen-DEAE 60-7, 7 µm, 125 x 4 mm, gradient 2 min 85% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 15% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0 until 30 min 25% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 75% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0): 20.8 min.

UV (20 mM $KH_2PO_4$ in $H_2O/CH_3CN$ 4:1, pH 6.0): 291.

MALDI-TOF-MS (Matrix ATT): calc. ddGlc[$UD_8$ + 7H]$^-$) 2649.7, found 2649.7.

**ddGlc[$^{4'}A_2DA_5{}^{6'}$]**

scale: 0.4 µmol

synthesis protocol: 1.0 µmol (0.05 M 2-(cyanoethyl)phosphoramidites)

coupling time: 1 min 30 s

$t_R$ (Nucleogen-DEAE 60-7, 7 µm, 125 x 4 mm, gradient 2 min 85% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 15% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0 until 30 min 25% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 75% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0): 23.1 min.

UV (20 mM $KH_2PO_4$ in $H_2O/CH_3CN$ 4:1, pH 6.0): 258.

MALDI-TOF-MS (Matrix ATT): calc. ddGlc[$A_2DA_5$ + 6H]$^-$) 2527.6, found 2527.5.

**ddGlc[$A_8$]**

scale: 0.4 µmol

synthesis protocol: 1.0 μmol (0.05 M 2-(cyanoethyl)phosphoramidites)

coupling time: 1 min 30 s

$t_R$ (Nucleogen-DEAE 60-7, 7 μm, 125 x 4 mm, gradient 2 min 85% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 15% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0 until 30 min 25% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 75% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0): 26.4 min.

UV (20 mM $KH_2PO_4$ in $H_2O/CH_3CN$ 4:1, pH 6.0): 257.

MALDI-TOF-MS (Matrix ATT): calc. ddGlc[$A_8$ + 6H]$^-$) 2553.6, found 2553.5.

**ddGlc[$^{4'}$(UA)$_5$$^{6'}$]**

scale: 0.4 μmol

synthesis protocol: 1.0 μmol (0.05 M 2-(cyanoethyl)phosphoramidites)

coupling time: 1 min 30 s

$t_R$ (*Nucleogen*-DEAE 60-7, 7 μm, 125 x 4 mm, gradient 2 min 85% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 15% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0 until 30 min 25% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 75% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0): 20.2 min.

UV (20 mM $KH_2PO_4$ in $H_2O/CH_3CN$ 4:1, pH 6.0): 258.

MALDI-TOF-MS (Matrix ATT): calc. ddGlc[$(UA)_5$ + 8H]$^-$) 3092.6, found 3092.6.

**ddGlc[$^{4'}$(UA)$_3$UD(UA)$_3^{6'}$]**

scale: 0.4 μmol

synthesis protocol: 1.0 μmol (0.05 M 2-(cyanoethyl)phosphoramidites)

coupling time: 1 min 30 s

$t_R$ (Nucleogen-DEAE 60-7, 7 μm, 125 x 4 mm, gradient 2 min 85% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 15% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0 until 30 min 25% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 75% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0): 24.4 min.

UV (20 mM $KH_2PO_4$ in $H_2O/CH_3CN$ 4:1, pH 6.0): 258.

MALDI-TOF-MS (Matrix ATT): calc. ddGlc[$^{4'}$(UA)$_3$UD(UA)$_3^{6'}$ + 12H]$^-$) 4328.9, found 4329.2.

**ddGlc[U$_8$]**

scale: 0.4 μmol

synthesis protocol: 1.0 μmol (0.05 M 2-(cyanoethyl)phosphoramidites)

coupling time: 1 min 30 s

$t_R$ (Nucleogen-DEAE 60-7, 7 μm, 125 x 4 mm, gradient 2 min 85% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 15% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0 until 30 min 25% 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0, 75% 1.0 M KCl, 20 mM $KH_2PO_4$ $H_2O/CH_3CN$ 4:1, pH 6.0): 9.7 min.

UV (20 mM $KH_2PO_4$ in $H_2O/CH_3CN$ 4:1, pH 6.0): 259.

MALDI-TOF-MS (Matrix ATT): calc. ddGlc[$U_8$ + 6H]$^-$) 2369.4, found 2369.2.

**ddGlc[$^{4'}$CAUA-X-GUGA$^{6'}$]**

scale: 0.4 µmol

synthesis protocol: 1.0 µmol (0.05 M 2-(cyanoethyl)phosphoramidites)

coupling time: 1 min 30 s

**ddGlc[$^{4'}$UCAC-X-UAUG$^{6'}$]**

scale: 0.4 µmol

synthesis protocol: 1.0 µmol (0.05 M 2-(cyanoethyl)phosphoramidites)

coupling time: 1 min 30 s

*Determination of oligonucleotide concentration in solution*: Oligo-homo-deoxyribonucleotide concentrations were determined in buffered, neutral solutions (pH 7.0) with UV-spectroscopy at 260 nm in the linear and nearly temperature independent region. For self-complementary sequences, the determination was done at a temperature higher than the melting point. The extinction coefficient was calculated as sum of all the extinction coefficients of all mononucleosides (at $\lambda$ = 260 nm), for which the following, experimentally determined ε-values were used: ddGlc(U) 10200, ddGlc(T) 9500, ddGlc(C) 7800, ddGlc(G) 11100, ddGlc(A) 14700, ddGlc(D) 670.

## *5.2 Determination of UV- and CD-melting curves*

UV- (resp. CD-)-melting curves were measured on a *Jasco J-715* spectrapolarimeter equipped with a *Julabo HS 18* temperature control unit. Solutions (0.15 M NaCl 0.01 TrisHCl buffer, pH 7.0) were heated and cooled in a linear way by 0.5-1° C/min and every 0.5° C (30-60 s)

CD/temperature resp. absorption/temperature (at 260 nm, otherwise noted) pairs were determined. The samples were degassed in a sonicator for 2 min before the measurement. Melting curves are reported in relative hyper- (resp. hypo-)chromism (in %) vs. temperature (in °C) or in fraction folded (θ) vs. temperature. The percentage of hyperchromism was determined according to the formula: $H = ((A(t)-A°)/A°) \times 100$, with $A(t)$ being the absorption at the temperature $t$ and $A°$ the lowest measured absorption (for a definition of hyper- (resp. hypo-)chromism see[84]). The fraction folded (θ) was determined according to a procedure described by *J.-L. Mergny* and *L.Lacroix*[85]: $\theta_T = (L0_T - A_T)/(L0_T - L1_T)$, with $L0_T$ and $L1_T$ being the baseline values of the unfolded and folded species and $A_T$ being the absorbance at a given temperature. $T_m$ values and thermodynamic data of duplex, single strand transitions were determined according to described methods.[83] *Van't Hoff* pairing enthalpies ($\Delta H$) and entropies ($\Delta S$) were determined by the linear correlation of the reciprocally $T_m$-values to the logarithm of the oligonucleotide concentration according to the formulas $1/T_m = (R/\Delta H) \ln c + \Delta S/\Delta H$ (bimolecular association of two self-complementary sequences) and $1/T_m = (R/\Delta H) \ln c + (\Delta S - R \ln 4)/\Delta H$ (biomolecular association of two non self-complementary sequences) with $c$ being the total oligonucleotide concentration and $R$ being the gas constant. An indication of the errors of the data was derived from the R-factors of the linear regression ($1/T_m$ vs. $\ln c$).

## *5.3 Crystallographic data*

| | |
|---|---|
| Crystallised from | $D_2O$ |
| Empirical formula | $C_{11}H_{17}N_3O_3$ |
| Formula weight [g mol$^{-1}$] | 239.27 |
| Crystal colour, habit | colourless, needle |
| Crystal dimensions [mm] | $0.05 \times 0.10 \times 0.30$ |
| Temperature [K] | 160(1) |
| Crystal system | monoclinic |
| Space group | $P2_1$ (#4) |
| $Z$ | 4 |

| | | |
|---|---|---|
| Reflections for cell determination | | 2852 |
| $2\theta$ range for cell determination [°] | | 4–55 |
| Unit cell parameters | $a$ [Å] | 10.4815(2) |
| | $b$ [Å] | 7.4459(1) |
| | $c$ [Å] | 15.5890(3) |
| | $\alpha$ [°] | 90 |
| | $\beta$ [°] | 108.579(1) |
| | $\gamma$ [°] | 90 |
| | $V$ [Å$^3$] | 1153.23(4) |
| $F$(000) | | 512 |
| $D_X$ [g cm$^{-3}$] | | 1.378 |
| $\mu$(Mo $K\alpha$) [mm$^{-1}$] | | 0.102 |
| Scan type | | $\phi$ and $\omega$ |
| $2\theta_{(max)}$ [°] | | 55 |
| Total reflections measured | | 26783 |
| Symmetry independent reflections | | 2859 |
| $R_{int}$ | | 0.090 |
| Reflections with $I > 2\sigma(I)$ | | 2342 |
| Reflections used in refinement | | 2859 |
| Parameters refined; restraints | | 334; 1 |
| Final $R(F)$ [$I > 2\sigma(I)$ reflections] | | 0.0486 |
| $wR(F^2)$ (all data) | | 0.1295 |
| Weights: | $w = [\sigma^2(F_o^2) + (0.0799P)^2 + 0.1709P]^{-1}$ where $P = (F_o^2 + 2F_c^2)/3$ | |
| Goodness of fit | | 1.042 |
| Secondary extinction coefficient | | 0.015(3) |
| Final $\Delta_{max}/\sigma$ | | 0.001 |
| $\Delta\rho$ (max; min) [e Å$^{-3}$] | | 0.30; -0.35 |
| $\sigma(d_{(C-C)})$ [Å] | | 0.004 |

**Table 6** Crystallographic Data

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